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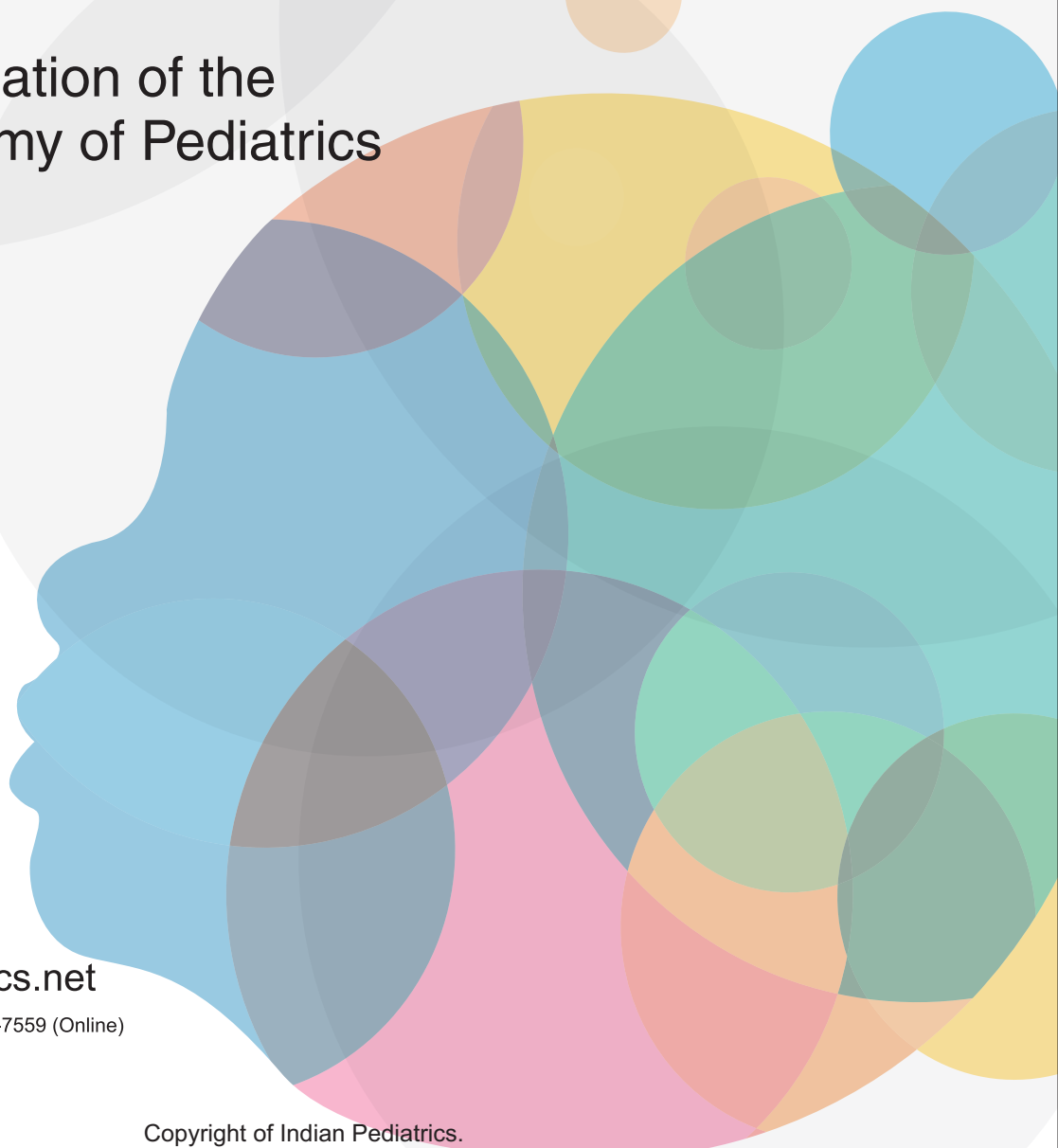

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Contents

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EDITORIAL COMMENTARY

Magnitude and Trends of Childhood Cancer in India

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India is a big country with a young population. Children and adolescents are nearly 40% of the population. Cancer in children and adolescents is highly curable. But barriers to cure remain in a country like India and first hurdle is diagnosing all children with cancer [1]. Reasons for no diagnosis or delayed diagnosis and referral are – *a*) insufficient political priority and funding from government, *b*) incorrect diagnosis and risk stratification, *c*) lack of diagnostic labs and centres for pediatric oncology, *d*) lack of trained manpower and training courses in pediatric oncology, and *e*) socio-economic and cultural factors [1]. New cases of childhood cancer in India have been quoted to be around 50,000 annually but on what basis this number was reached in not known. Is this number right? In this issue, Arora, et al. give us a fresh perspective and new numbers of around 52,366 annually much higher than from previously predicted number of 28,712 annually [2]. If we include adolescents also then annual number is expected to reach 76,805 [2]. There is a huge gap (>50%) in perceived or agreed number and previously predicted number, leading to suggestions that childhood cancer diagnosis is being missed. Reaching the unreached is the new mantra to improve outcomes of childhood cancer. Lack of integrated national health service and referral system is the main reason why childhood cancer patients are being missed. Training of primary care doctors and nurses in suspecting cancer is needed and improving diagnostics and reducing cost of tests can improve this.From certain geographical areas of India (mountains, north east, islands, desert etc); its difficult to collect data. More males are diagnosed with childhood cancer then females a stark finding in India [2]. Gender gap >10% is possibly explained by gender bias in the Indian society.

Knowing the full burden of cancer is a must for government to allocate resources. Making cancer a notifiable disease can help in capturing the exact numbers. Few states like Punjab, have already done this [1].There is a need for national cancer registry for the same [3]. Linking all medical colleges and post graduate training institutes and cancer centers in a national grid/registry can help collect this data. Cancers like leukemia, which are easier to diagnose by simple blood tests or bone marrow tests, are picked up more easily and brain tumors are either not diagnosed or diagnosed late because it needs imaging like CT scan or MRI, which are not easily available [2]. However, progress has been made on all fronts in the last few decades to improve outcomes of childhood cancer in India, especially acute lymphoblastic leukemia [4].

To know the trends of childhood cancer we need long-term data. Delhi is big city and quite representative of India with mixed rural and urban population. In this issue, Malhotra, et al. [5] describe patterns and trends of childhood cancer in Delhi. Over 25 years, on an average 500 cases/year were diagnosed, which is in agreement with numbers predicted by Arora, et al. for Delhi [2]. Childhood cancer has increased by nearly 100% over these last 25 years in Delhi [5]. Pesticides and pollution may have a link and studies are needed to prove it. Does religion have connection? Does diet have an impact? Does education have an impact? A study reported incidence of cancer in children of different religions is similar to that of proportions of population of different religions in Delhi except for Jain religion where incidence of cancer is 6 times higher despite lesser number children in 0-6 year in this population as compared to other religions. Major difference is the dietary practices, but another reason could be increased literacy levels in Jain community improving chances of better access to care [6]. Data regarding comprehensive childhood cancer burden in country is lacking due to low, and urban predominant coverage of population-based cancer registry programs. The childhood cancer services in India are predominantly restricted to few tertiary care centres in major cities [7]. Certain specialized treatments like eye enucleation, radiotherapy or limb salvage surgery are not available at all the centers. This may be the reason for higher reporting of retinoblastoma and bone tumors from Delhi registry [5,7]. In a survey of 20 pediatric cancer units across the country; approximately 3500 childhood cancer cases were diagnosed annually. Top five cancers reported were leukemia-34%, brain tumors-

10%, lymphoma-10%, bone tumors-9% and retinoblastoma-5% [8]. These findings are similar to Delhi registry [5] but again highlight that brain tumors are underdiagnosed.

To improve the cure of childhood cancer in India; the very first step is establishing a national cancer registry and improving diagnosis and referral of children with cancer. Both professionals and the government need to step up for this onerous task.

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RESEARCH PAPER

Estimated National and State Level Incidence of Childhood and Adolescent Cancer in India

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Background: Hitherto, incidence burden of childhood cancer in India has been derived from GLOBOCAN data. Recent analyses have challenged whether this accurately measures the true incidence of childhood cancer.

Objective: To use observed data rather than simulation to estimate the number of children (0-14 years), as well as number of children and adolescents (0-19 years), in India who develop cancer every year at the national and state/union territory (UT) level.

Methods: Age-specific (five year groups), sex-specific, and state/UT specific population data from India Census 2011 was used. Global average incidence rates from the International Incidence of Childhood Cancer 3 (IICC3) report were used. Incidence rates per million person-years for the 0-14 years and 0-19 years age groups were age-adjusted using the world standard population to provide age-standardized incidence rates, using the age-specific incidence rates for individual age groups (0-4 years, 5-9 years, 10-14 years, and 15-19 years).

Results: The national number of children (0-14 years) and, children and adolescents (0-19 years) that may develop cancer every year based on 2011 census are 52,366 and 76,805 persons respectively. Cancer type specific incidence is provided for each state/UT for these age ranges. This national incidence is approximately double of the GLOBOCAN 2018 estimates of incidence of children diagnosed and registered with cancer and the differential is greater in girls.

Conclusion: Our analysis proposes new estimates of incident childhood cancer cases in India for children and adolescents. Future regional, national and international research on childhood cancer epidemiology and healthcare accessibility would help further refine these estimates.

Keywords: *Cancer registry, Epidemiology, Incidence, Population data.*

Defining the local incidence of cancer is a key first step towards developing a comprehensive cancer control strategy [1]. In the context of childhood cancer, such information helps to understand disease etiology, improve access to care, plan investments in service delivery, advocate resource allocation, and measure the quality of different components of the health system [1].

Estimates of global and country-specific cancer and childhood cancer burden are provided by multiple groups. The recently published GLOBOCAN 2018 study [2], coordinated by the International Agency for Research on Cancer, provides comprehensive global childhood cancer incidence estimates and is commonly used by the World Health Organization and governments for planning cancer control. In 2018, the study estimated that 200,166 new children, age 0-14 years, were diagnosed and registered with cancer globally, of whom 28,712 (14.3%) were from India [2].

Recent analyses have questioned the accuracy of GLOBOCAN data for estimating the incidence of childhood cancer [3]. The local incidence of childhood cancer varies substantially in the published data including that from India [4,5]. It has been hypothesized that under-diagnosis and consequently under-registration, which is disproportionately high in low and middle income countries (LMIC), leads to an "incidence gap" and under-estimates the cancer burden, and are hence not reflected in the GLOBOCAN 2018 data [6]. This theory has been further substantiated by independent simulation-based studies that have estimated the annual global childhood cancer burden is nearly 45% greater than that historically reported, between 360,000 to 400,000, when children who develop cancer but are never registered are counted [7,8].

Editorial Commentary: Pages 415-16

Due to perceived incomplete case-finding, misdiagnosis within the fragmented Indian health system and significantly lower incidence-rates of childhood cancer in India, the currently reported childhood cancer from GLOBOCAN 2018 likely represent an under-estimate [5,9]. In this study, we aim to use observed data

rather than simulation to estimate the number of children (0-14 years), as well as number of children and adolescents (0-19 years), in India who develop cancer every year. Additionally, we report these data at the national and state/union territory (UT) level for the purposes of supporting cancer control planning.

METHODS

Age-specific (five year groups), sex-specific, and state/UT- specific population data from India Census 2011 was used [10]. These data pre-date the division of Andhra Pradesh in 2014 and Jammu and Kashmir in 2019 and hence considers these states as a whole. Conducted every 10 years since 1872, phase one of the 2011 census began on 1st April 2010 and included house-listing and collecting information for the National Population Register. The second phase was the population enumeration phase done from 9 to 28 February, 2011.

Global average incidence rates from the International Incidence of Childhood Cancer 3 (IICC3) report were used [4]. Conducted by the International Agency for Research on Cancer with the specific purpose of collecting and disseminating childhood cancer data, IICC-3 is the third monograph following from IICC-1 published in 1988 and IICC-2 published in 1998. Only population based cancer registries were invited. The target period covered the years starting with 1990, and targeted the age range of 0-19 years. IICC-3 uses observed data on cancer incidence from countries or regions covered by population-based cancer registries and unlike GLOBOCAN does not extrapolate to produce selected national, regional or global cancer burden estimates.

Incidence rates per million person-years for the 0-14 years (children) and 0-19 years (children and adolescents) age groups were age-adjusted using the world standard population to provide age-standardised incidence rates, using the age-specific incidence rates for individual age groups (0-4 years, 5-9 years, 10-14 years, and 15-19 years).

Statistical analyses: Number of incident cases for 0-14 years, 0-19 years and individual age groups (0-4 years, 5-9 years, 10-14 years, and 15-19 years) was calculated by multiplying incidence rates with the denominator population for the country and each state/UT. To get cancer-specific incident cases according to the International Childhood Cancer Classification third edition in 0-14 years age group, cancer-specific incidence rates were multiplied with the denominator population for the country and each state/UT [11]. As cancer-specific incidence rates were not available for 0-19 year age group, cancer-specific incident cases for this age group were obtained by adding incident cases in the 0-14 year age group derived above and cancer-specific incident cases in the 15-19 year age group. To derive the cancer-specific incident cases in 15-19 year age group, cancer-specific incidence rates for this agre group were multiplied with the denominator population for the country and each state/UT.

RESULTS

Using globally observed data and local population estimates, the national number of children (0-14 years) and, children and adolescents (0-19 years) that may develop cancer every year are based on 2011 census as 52,366 and 76,805 persons, respectively (**Table I**). The national incidence for boys and girls of 0-14 years of age are 29,425 and 23,045 persons, respectively, and 42,160 boys and 33,694 girls for those 0-19 years of age. Uttar Pradesh, Bihar, Maharashtra, West Bengal and Madhya Pradesh are the five states with the largest absolute burden of disease (**Table I**). Leukemias, central nervous system (CNS) tumors and lymphomas are the three most common cancers in the 0-14 years age group contributing to 33.0%, 20.1% and 10.8% of the total burden (**Table II**), and account for 27.0%, 16.8% and 13.9%, respectively of the total burden in the 0-19 years age group (**Table III**).

DISCUSSION

The National Cancer Registry Program (NCRP) in India provides data for the observed individual population based cancer registries which include all patients with cancer diagnosed and registered, and cover less than 10% of the Indian population [12]. The NRCP report, however, does not extrapolate to provide an estimate of the national incidence of childhood cancer. National estimates used for cancer control planning in India are provided by the GLOBOCAN 2018 models that are built using individual cancer registry data from the NCRP report, national vital statistic data sets and economic development covariates [2,12]. In this analysis, using internationally standardized incidence rates and population-estimates from India, we found that the incidence of childhood cancer is 54.8% larger in 0 to 14 years age range (52366 vs 28712) and 50.3% larger in 0 to 19 years age range (76805 vs 38640) compared to GLOBOCAN 2018. We hypothesize the large observed difference between the two estimates is due to the substantial number of cases that are not diagnosed and/or registered in India [6-8].

For health systems planning, calculating both the number of patients who will develop cancer and the

Table I Age- and Gender-Specific Incident Cases of Cancer in Children and Adolescent in States and Union Territories of India

	0-4 y	*5-9 y*	*10-14 y*	*15-19 y*	*0-14 y, boys*	*0-14 y, girls*	*0-14 y, both*	*0-19 y, boys*	*0-19 y, girls*	*0-19 y, both*
Incidence rate (per million)	187.9	107.6	114.4	185.3	151.4	129.4	140.6	163.2	143.6	155.8
India	21196	13657	15182	22334	29425	23045	52366	42160	33694	76805
Andaman & Nicobar	5	3	4	6	7	6	13	11	9	20
Andhra Pradesh	1181	786	938	1500	1696	1370	3064	2514	2080	4656
Arunanchal Pradesh	27	18	21	29	38	32	69	54	46	101
Assam	604	381	399	569	792	649	1441	1114	932	2075
Bihar	2399	1618	1592	1755	3285	2591	5866	4404	3476	7976
Chandigarh	15	10	11	19	22	16	37	33	24	58
Chhattisgarh	477	297	330	482	629	522	1151	893	763	1680
Dadra & Nagar Haveli	7	4	4	6	9	7	15	12	9	22
Daman & Diu	4	2	2	5	4	3	8	8	5	13
Delhi	260	165	189	309	372	273	642	552	409	971
Goa	19	11	13	21	25	20	45	37	30	67
Gujarat	1026	628	703	1087	1405	1056	2453	2028	1563	3632
Haryana	444	269	305	496	625	440	1059	917	659	1590
Himachal Pradesh	102	64	73	119	142	109	250	207	164	376
Jammu & Kashmir	266	152	162	229	341	257	596	473	371	853
Jharkhand	686	445	470	594	924	749	1672	1276	1045	2352
Karnataka	948	564	656	1080	1247	1008	2253	1840	1519	3405
Kerala	461	275	323	484	604	497	1101	868	736	1627
Lakshawdeep	1	1	1	1	1	1	2	2	2	3
Madhya Pradesh	1404	889	980	1380	1911	1511	3417	2710	2175	4946
Maharashtra	1759	1057	1228	1969	2389	1829	4206	3507	2736	6317
Manipur	48	31	36	53	67	54	121	96	80	179
Meghalaya	76	42	44	60	90	75	166	124	107	234
Mizoram	23	13	13	20	27	23	50	39	33	73
Nagaland	37	25	28	43	53	42	95	77	63	142
Odisha	686	438	497	727	934	765	1698	1328	1129	2493
Puducherry	18	11	12	19	23	19	42	33	28	62
Punjab	401	255	296	522	590	412	996	892	637	1543
Rajasthan	1372	865	959	1355	1900	1446	3336	2683	2097	4836
Sikkim	8	6	8	12	13	11	23	19	16	36
Tamil Nadu	992	597	707	1159	1325	1068	2391	1956	1619	3624
Tripura	61	36	41	66	79	64	143	114	97	214
Uttar Pradesh	3829	2697	2960	4269	5691	4363	10026	8143	6383	14700
Uttarakhand	174	114	131	208	250	191	440	366	289	663
West Bengal	1378	887	1048	1678	1913	1566	3478	2830	2363	5265

Table II International Childhood Cancer Classification Type-Specific Incident Cases of Cancer in Children 0-14 Years of Age in States and Union Territories of India

	Leukemias	*Lymphomas*	*CNS tumors*	*SNS tumors*	*Retino-blastoma*	*Renal tumors*	*Hepatic tumors*	*Bone tumors*	*Soft tissue sarcomas*	*Germ cell tumors*	*Epithelial tumors* [a]	*Other* [b]
Incidence rate (per million)	46.4	15.2	28.2	10.4	4.5	8.2	2.3	5.7	8.9	4.9	4.6	1.2
India	17281	5661	10503	3873	1676	3054	857	2123	3315	1825	1713	447
Andaman & Nicobar	4	1	3	1	0	1	0	1	1	0	0	0
Andhra Pradesh	1011	331	615	227	98	179	50	124	194	107	100	26
Arunanchal Pradesh	23	7	14	5	2	4	1	3	4	2	2	1
Assam	476	156	289	107	46	84	24	58	91	50	47	12
Bihar	1936	634	1177	434	188	342	96	238	371	204	192	50
Chandigarh	12	4	8	3	1	2	1	2	2	1	1	0
Chhattisgarh	380	124	231	85	37	67	19	47	73	40	38	10
Dadra & Nagar Haveli	5	2	3	1	0	1	0	1	1	1	0	0
Daman & Diu	3	1	2	1	0	0	0	0	0	0	0	0
Delhi	212	69	129	47	21	37	11	26	41	22	21	5
Goa	15	5	9	3	1	3	1	2	3	2	1	0
Gujarat	809	265	492	181	79	143	40	99	155	85	80	21
Haryana	349	114	212	78	34	62	17	43	67	37	35	9
Himachal Pradesh	82	27	50	18	8	15	4	10	16	9	8	2
Jammu & Kashmir	197	64	120	44	19	35	10	24	38	21	20	5
Jharkhand	552	181	335	124	54	98	27	68	106	58	55	14
Karnataka	744	244	452	167	72	131	37	91	143	79	74	19
Kerala	363	119	221	81	35	64	18	45	70	38	36	9
Lakshawdeep	1	0	0	0	0	0	0	0	0	0	0	0
Madhya Pradesh	1128	369	685	253	109	199	56	139	216	119	112	29
Maharashtra	1388	455	844	311	135	245	69	171	266	147	138	36
Manipur	40	13	24	9	4	7	2	5	8	4	4	1
Meghalaya	55	18	33	12	5	10	3	7	10	6	5	1
Mizoram	17	5	10	4	2	3	1	2	3	2	2	0
Nagaland	32	10	19	7	3	6	2	4	6	3	3	1
Odisha	560	184	341	126	54	99	28	69	107	59	56	14
Puducherry	14	5	8	3	1	2	1	2	3	1	1	0
Punjab	329	108	200	74	32	58	16	40	63	35	33	9
Rajasthan	1101	361	669	247	107	195	55	135	211	116	109	28
Sikkim	8	3	5	2	1	1	0	1	1	1	1	0
Tamil Nadu	789	259	480	177	77	139	39	97	151	83	78	20
Tripura	47	15	29	11	5	8	2	6	9	5	5	1
Uttar Pradesh	3309	1084	2011	742	321	585	164	406	635	349	328	86
Uttarakhand	145	48	88	33	14	26	7	18	28	15	14	4
West Bengal	1148	376	698	257	111	203	57	141	220	121	114	30

CNS – central nervous system, SNS – sympathetic nervous system. [a] *also includes melanomas,* [b] *also includes unspecified.*

Table III International Childhood Cancer Classification Type-Specific Incident Cases* of Cancer in Children 0-19 Years of Age in States and Union Territories of India

	Leukemias	*Lymphomas*	*CNS tumors*	*SNS tumors*	*Retino-blastoma*	*Renal tumours*	*Hepatic tumors*	*Bone tumors*	*Soft tissue sarcomas*	*Germ cell tumors*	*Epithelial tumors* [a]	*Other* [b]
India	20716	10699	12901	3958	1676	3223	1001	3859	4870	4501	6474	784
Andaman & Nicobar	5	3	3	1	0	1	0	1	1	1	2	0
Andhra Pradesh	1242	670	776	232	98	190	60	241	298	286	420	49
Arunanchal Pradesh	27	14	17	5	2	4	1	5	6	6	8	1
Assam	563	284	350	109	46	88	27	103	131	118	168	21
Bihar	2206	1030	1365	441	188	355	107	374	494	415	566	77
Chandigarh	15	8	10	3	1	2	1	3	4	4	5	1
Chhattisgarh	454	233	283	87	37	71	22	84	106	98	140	17
Dadra & Nagar Haveli	6	3	4	1	0	1	0	1	1	1	2	0
Daman & Diu	3	2	2	1	0	0	0	1	1	1	1	0
Delhi	259	139	162	49	21	40	13	50	62	59	87	10
Goa	18	10	11	3	1	3	1	3	4	4	6	1
Gujarat	977	510	609	186	79	151	47	184	231	216	312	37
Haryana	426	226	266	80	34	65	21	81	102	96	140	17
Himachal Pradesh	101	54	63	19	8	15	5	19	24	23	33	4
Jammu & Kashmir	232	116	144	45	19	37	11	42	54	48	68	9
Jharkhand	643	315	399	126	54	102	31	114	147	129	181	23
Karnataka	910	487	568	171	72	140	44	175	218	208	304	36
Kerala	438	228	273	83	35	68	21	82	103	96	139	17
Lakshawdeep	1	0	1	0	0	0	0	0	0	0	0	0
Madhya Pradesh	1340	681	834	258	109	210	65	246	312	284	406	50
Maharashtra	1691	899	1055	319	135	260	82	324	403	383	557	66
Manipur	48	25	30	9	4	7	2	9	11	11	15	2
Meghalaya	64	32	40	12	5	10	3	11	15	13	18	2
Mizoram	20	10	12	4	2	3	1	4	5	4	6	1
Nagaland	38	20	24	7	3	6	2	7	9	8	12	1
Odisha	672	348	419	128	54	105	32	125	158	146	211	25
Puducherry	17	9	10	3	1	3	1	3	4	4	5	1
Punjab	409	225	256	76	32	62	20	81	99	97	144	16
Rajasthan	1309	666	815	252	107	205	63	241	306	279	398	49
Sikkim	10	5	6	2	1	1	0	2	2	2	3	0
Tamil Nadu	967	520	604	181	77	148	47	187	232	222	325	38
Tripura	57	30	36	11	5	9	3	11	14	13	19	2
Uttar Pradesh	3965	2047	2469	758	321	617	192	738	932	861	1238	150
Uttarakhand	177	95	111	33	14	27	9	34	42	40	59	7
West Bengal	1406	755	878	264	111	216	68	271	337	322	472	55

**Incidence rate for each of the major cancer types for the 0-19 years age group were not available and the burden was estimated by adding incident cases in the 0-14 year age group and 15-19 year age group (incidence rates for the cancer subtypes for the ages 15-19 were available); CNS: central nervous system; SNS: sympathetic nervous system.[a] also includes melanomas;[b]also includes unspecified*

WHAT IS ALREADY KNOWN?

- Incidence burden of childhood cancer in India has been derived from GLOBOCAN data.

WHAT THIS STUDY ADDS?

- The national number of children (0-14 years), and children and adolescents (0-19 years) that may develop cancer every year in India (based on census 2011) are 52366 and 76805 persons, respectively.
- This is approximately double the previous estimates of incidence of children diagnosed and registered with cancer.

number of patients who are diagnosed and registered is critical information. Knowing the current healthcare utilization needs presently is critical for states to make allocation decisions today. However, as cancer control plans typically are written as multi-year plans, identifying the gap between the observed and expected cases is important. In particular, as strategies to improve access and referral are often built into national cancer control plans, these calculations can inform prioritization, decision-making, monitoring procedures and budgeting.

Not only is the incidence of diagnosed and registered (GLOBOCAN 2018) approximately half of those who develop cancer (our estimates), **Suppl. Table I** shows this differential varies by age, gender and cancer. The estimated proportion of girls diagnosed and registered with cancer is 10% less than boys. This aligns with the narrative of female children with cancer experiencing relatively greater barriers to accessing healthcare [5,13-15]. Similarly the differential of the GLOBOCAN 2018 estimates and those from our analysis is greatest in CNS tumors and lowest in leukemias. This may reflect the relatively sick nature of leukemia patients, and easy availability of automated blood counts and bone marrow examination as compared to more sophisticated and technology dependent interventions like neuroimaging and neurosurgery. There is also a component of under-ascertainment in diagnosed CNS tumors as currently NCRP datasets exclude tumors with 'benign' or 'uncertain' behavior and such tumors constitute 40-50% of CNS tumors in children and adolescents [16].

Limitations of our analysis are that we are using the 2011 census data and hence have likely slightly over-estimated the incidence of new cases. Although the population of India is projected to peak around 2050, that for children ages 0-19 years is expected to peak between 2010 to 2020. And hence one can argue that the burden in 2011 will be higher by a few percentage points than the burden in 2020 and beyond. The census 2011 however remains the most reliable estimates of population at the state and union territory level and hence was used. It is also difficult to be more precise to the relative contributions of under-diagnosis versus under-registration although there is some evidence to support that under-diagnosis is the main component of 'incidence gap' in the burden [17]. The contribution of under-diagnosis and under-registration may vary across states depending on the healthcare accessibility but in our analysis we have assumed that it is same across states.

Perhaps the most important question in regard to our estimates is its reliability and accuracy. While there is a degree of uncertainty around the burden, its reliability can be inferred from two arguments. Firstly, is the central tenet that environment plays a minor role in the etiology of childhood cancer hence the variation in the incidence of childhood cancer across the world is limited [4,18]. Secondly, under-diagnosis and other aspects of impaired healthcare access like delayed diagnosis, abandonment of treatment, etc. are well-recognized issues in LMICs [5,14,17,19,20]. Our estimates of 45-50% under-diagnosed children mirrors other recently published data which reached similar conclusions using differing methodologies [7,8].

In conclusion, our analysis proposes new estimates of incident childhood cancer cases in India. We also provide estimates at state and union territory level. This has enormous implications for all childhood cancer stakeholders who aim to provide access, treatment and chance of long-term cure to every child with cancer. It also suggests that access to diagnosis is as big, if not a bigger problem, than access to complete treatment and needs to be tackled early and urgently. Future regional, national and international research on childhood cancer epidemiology and healthcare accessibility would help further refine these estimates.

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Supplementary Table I Comparison of Childhood Cancer Burden Estimates by GLOBOCAN and Our Analysis

	0 to 14 years age			0 to 19 years age		
	Globocan	Our Estimate	% Diagnosed & Registered	Globocan	Our Estimate	% Diagnosed & Registered
Total	**28712**	**52366**	**54.8**	**38640**	**76805**	**50.3**
Boys	17468	29425	59.4	22960	42160	54.5
Girls	11244	23045	48.8	15680	33964	46.2
Leukemia*	11056	17281	64.0	13637	20716	65.8
Lymphoma*	3591	5661	63.4	5019	10699	46.9
CNS tumours*	3626	10503	34.5	4638	12901	36.0
Kidney tumours*	1466	3054	48.0	1578	3223	49.0
Liver tumours*	421	857	49.1	481	1001	48.1

*Only those cancers were selected where the ICD site classification aligns closely with the ICCC morphology classification

CNS – central nervous system

RESEARCH PAPER

Reference Ranges of Different Lymphocyte Subsets in Indian Children: A Multi-Centric Study

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Objective: To determine the reference ranges of various lymphocyte subsets in healthy Indian children.

Design: Descriptive cross-sectional study.

Setting: Four centers in India representing four geographical regions.

Participants: 1104 children from neonatal age to 18 years of age.
Measurement: One time measurement of absolute count and percentages of different lymphocyte subsets i.e. T lymphocytes (CD3+T, CD4+T, CD8+T cells), B lymphocytes (CD19+B cells) and Natural Killer lymphocytes (CD15/16+NK cells) in whole blood using multicolor flow cytometry.

Results: The absolute cell counts of various lymphocytes were found to increase from newborn to 10 months of age, followed by gradual decline until 18 years; however, the proportion of immune cells remained largely similar. Gender did not have a significant impact on the reference ranges, whereas counts were found to vary as per the geographical locations.

Conclusions: These reference ranges will be useful to monitor and predict the immune status in pediatric population. The variation in region wise ranges could be confirmed by testing more number of samples in the specific age groups.

Keywords: *Flow cytometry, NK cell, CD4+T cells, B cells.*

Cellular differentiation pathways in children are distinctly different form adults [1]. Additionally, the cellular immune component of the blood is known to be dynamic and showing variable frequencies of different immune subsets at different ages especially in pediatric population [7]. In India, although the lymphocytic reference ranges are available for healthy adults [2], there is not much data available on the reference range of lymphocytic subsets among pediatric population [3]. Since the ethnicity, age and environmental factors are known to influence the lymphocytic reference ranges [4-6], the available reference ranges from other countries cannot be used for the Indian population. Considering the variations in ethnicity across various geographic regions in India, it is important to generate the reference values in different pediatric age groups from across the country

In this study, we determined the age group specific values for major lymphocyte subsets among healthy pediatric population aged from newborn to 18 years across different geographical regions in India.

METHODS

This cross-sectional study was aimed at determining the reference values of lymphocyte subsets in healthy Indian children aged 0 through 18 years from four geographically diverse sites i.e., Bengaluru, Chandigarh, Mumbai and Kolkata, in order to obtain the data representative of the entire country. The different age groups included in the study were: Group I- Newborn; Group II - 6 weeks of age (before first DPT vaccination); Group III - 9 to 10 months of age (before measles vaccination); Group IV - 15 to 18 months of age or before first booster of DPT; Group V - 19 months to 5 of age, Group VI - > 5 to 12 years of age; and, Group VII - 12 to 18 years of age. The immunization visits coincided with the blood collection visits.

For group I, cord blood was used as a sample. For this group, babies with full term normal vaginal delivery or elective caesarean with or without mild anemia in pregnancy were included. Emergency caesarean cases, complicated deliveries with chronic illness or with infections, conditions like diabetes, toxemia, bleeding,

fever in mother, prolonged rupture of membrane, and HIV positive pregnancy were excluded from this study.

For other groups (Group II to VII), the inclusion criteria for healthy children in the different age group were: no history of cold and cough (for the last one month), blood transfusion (preceding 3 months), surgery (preceding 6 months) and recent diarrhea (4-6 weeks), born to HIV negative mother, and grade 1 malnutrition/ normal weight (weight-for-age >70th centile IAP chart). Children with moderate to severe anemia, acute or chronic infectious diseases (gastrointestinal diseases within the last 6 months) or any clinically significant disease or findings in the medical history that might compromise the study measures (e.g., diabetes mellitus, asthma, rheumatoid arthritis, cystitis fibrosis) were excluded from the study.

Children were enrolled after obtaining written consent from their parents, and assent, if required. The study was approved by the ethics committees of respective study sites. From each site, 50 children were enrolled in each group and in each age group an attempt was made to enroll boys and girls in a 1:1 ratio. The enrollment for groups I, II, III and IV was done in the hospitals (well-baby clinics of the hospitals) and for groups V, VI and VII, school-going healthy children were enrolled after obtaining appropriate permission from the school. In one day, not more than 5-7 eligible newborns/ infants/ children were enrolled in each group (Groups I to IV). For the groups V, VI and VII, the schools were contacted and the eligible participants were enrolled sequentially. The data on age, sex, place of origin, height, weight, nutritional status and vaccination was collected wherever possible.

Two to five milliliter of whole blood specimens were collected from the children in K3 EDTA evacuated tubes and were processed for immune-phenotyping the same day. To avoid diurnal variation, the samples were uniformly collected in the forenoon at all the study sites.

Immunophenotyping: The enumeration of different lymphocyte subsets were done by multicolor flow cytometry. The single platform technology was used to obtain both the absolute counts and percentages. All the centers used the same reagents, equipments and the standard operating procedure to obtain comparable data. Briefly, in the two separate Trucount tubes, 50 µL of whole blood and 20 µL of liquid antibody reagents (CD3 FITC, CD8 PE, CD45 PerCP, CD4 APC) or (CD3 FITC, CD16+56 PE, CD45 PerCP, CD19 APC) was added. All reagents were from the Becton Dickinson. The tubes were incubated at room temperature in dark for 15 minutes. Lysis of the red blood cells was carried out using 450 µl of 1:10 diluted FACS lysing solution). A total of 100000 cells were acquired in a FACSCalibur (BD Bio-sciences) and analyzed using Multiset software (BD Biosciences). The absolute count and percentage of the lymphocyte subsets in the gate $CD45_{high}/SSC_{low}$ i.e., the count or the percentage from the total lymphocyte population was calculated by the Multiset software. B lymphocytes were identified as CD19+, T lymphocytes as CD3+ and further differentiated as CD4+ and CD8+ T cells and NK cells were identified as CD3-CD16/CD56+cells

The optical alignment of the equipment and fluorescence compensation settings were ensured daily by running the calibration beads (CaliBRITE 3) and the compensation was done using the FACSComp software. Additionally, each center successfully participated in National external proficiency testing programmed for CD4 count estimation.

Data analyses: To determine normal ranges of lymphocyte parameters, 2.5 and 97.5 percentile values were calculated, which covers 95% of the population [8]. The age, gender and region specific ranges were also reported. Any differences in lymphocyte subsets within the geographic regions were assessed using Kruskal-Wallis Chi square test. The region-wise value for each parameter in each age group were compared with the overall reference range of the respective parameter using Mann-Whitney U test. *P* value of <0.05 was considered as significant. Analyses were done using IBM SPSS 24.0.

RESULTS

A total of 1674 children were enrolled across the four regions. Of these, data collected from 1104 subjects was considered for analysis; 570 samples were excluded due to various reasons like quality of samples, fail to fit in hemoglobin, BMI or weight criterion etc. Region- and age-wise numbers of subjects in each group are shown in **Table I**. The representation of the samples in groups II (6.1%) and III (11.3%) were lower as compared to the other groups. The median (range) weight and hemoglobin of the newborns was 3 (2.5-4.5) kg and 16.1(13-22.1) g/ dL, respectively. The hemoglobin decreased to 11.8 (11-15.1) g/dL in group II, but remained similar in older age groups. The median (range) body mass index was 16.6 (13.9-19.6) in group II, which remained similar in older age groups.

The median and 2.5th and 97.5th percentiles of absolute counts and frequencies (% populations) of various lymphocyte subsets; CD3+, CD4+ and CD8+ T cells, B cells and NK cells in seven different age groups are presented in **Table II** and **Fig. 1**.

Table I Regional Distribution of the Study Participants (*N*=1104)

	East *n=317*[a]	*North* *n=281*[b]	*South* *n=304*[c]	*West* *n=202*[d]
Newborn, *n*=194	50 (25.8)	51 (26.3)	49 (25.3)	44 (22.7)
6-32 wk, *n*=67	23 (34.3)	39 (58.2)	0	5 (7.5)
9-10 mo, *n*=125	49 (39.2)	24 (19.2)	49 (39.2)	3 (2.4)
15-18 mo, *n*=132	46 (34.8)	28 (21.2)	47 (35.6)	11 (8.3)
19 mo-5 y, *n*=197	54 (27.4)	44 (22.3)	50 (25.4)	49 (24.9)
5-12 y, *n*=210	44 (21)	55 (26.2)	59 (28.1)	52 (24.8)
12-18 y, *n*=17[a]	51 (28.5)	40 (22.3)	50 (27.9)	38 (21.2)

No. of boys in each region: [a]*176,* [b]*138,* [c]*145 and* [d]*101.*

The absolute counts of CD3+, CD8+, CD4+ T cells and CD19+B cells increased during the first few months till 9-10 months and decreased gradually from 15-18 months onwards till 12-18 years while NK cells showed a gradual decline in the absolute count post 6 weeks of birth till 15-18 months of age and then plateaued (**Fig. 1a**). The percentage values of CD3+, CD8+, CD4+ T cells (**Fig. 1b**) along with the ratio of CD4 and CD8 largely remained unchanged across different pediatric age groups. The percentage of CD19+ B cells however increased from 6 weeks to 5 years and later decrease in age group of 5-12 years and further in 12-18 years of age. The percentage of NK cells started to decline from 6 weeks onwards till 5 years of age and later increased and reached to the levels present in new born babies (**Fig. 1b**).

The female: male ratio was similar across different age groups (range: 1: 0.9 to 1: 1.19). The age-specific overall ranges (**Table II**) were compared with the gender-wise ranges in each age group for each parameter. We found no significant difference in the reference values observed in male and female children for any parameter in any age group.

Similarly we also compared the region-wise reference values with the overall reference ranges within every age group for each parameter. The significant difference was observed in case of group I (newborn), group IV (15th 18 months of age), group V (19 months to 5 years), group VI (5 years to 12 years) and group VII (12 years to 18 Years) for a few parameters. In newborn group, the values were

Table II Median and Reference Range of Different Immune Cells in Indian Healthy Children of Different Age Groups (*N*=1104)

	Newborn *n*=194	*6 -32 wk* *n*=67	*9 -10 mo* *n*=125	*15-18 mo* *n*=132	*19 mo-5 y* *n*=197	*5-12 y* *n*=210	*12-18 y* *n*=179
CD3+cells							
Absolute counts	2731 (979-5024)	3421 (952-8586)	4630 (1623-8159)	3801 (1480-6475)	3110 (1191- 6692)	2347 (1191-4497)	1960 (1035-4493)
Percentage	65 (42-85)	63 (38-78)	63 (45-76)	64 (37-77)	63 (51-75)	67 (51-77)	66 (54-89)
CD4+cells							
Absolute counts	1827 (601-3243)	2156 (659-6132)	2852 (913-5680)	2271 (817-4893)	1821 (794-4323)	1266 (618-2555)	1080 (582-2045)
Percentage	43(23-58)	44 (15-60)	39 (24-58)	38 (24-53)	39 (26-50)	37 (26-50)	36 (26-520)
CD8+cells							
Absolute counts	881 (337-1889)	970 (159-3717)	1407 (455-3393)	1319 (549-2844)	1084 (315-2258)	913 (422-1878)	767 (405-2615)
Percentage	20 (11-40)	18 (7-42)	20 (10-37)	22 (10-40)	23 (13-32)	26 (17-38)	26 (17-52)
CD4/ CD8 ratio	2.0 (0.8-4.6)	2.4 (0.5-7.0)	2.0 (0.8-5.5)	1.7 (0.6-3.7)	1.8 (0.9-3.3)	1.4 (0.8-2.4)	1.4 (0.6-2.4)
CD19+cells							
Absolute counts	760 (70-2532)	1654 (351-5946)	1915 (523-3799)	1484 (246-4139)	1187 (362-2754)	653 (295-1650)	507 (115-1117)
Percentage	18 (4-43)	27 (11-44)	27 (13-42)	26 (8-42)	25 (16-37)	19 (11-33)	18 (4-29)
CD16+/56+ cells							
Absolute counts	499 (125)	489 (114-1624)	433 (105-1088)	368 (114-1201)	335 (131-1163)	362 (124-1005)	334 (78-774)
Percentage	12 (4-36)	8 (2-18)	6 (2-16)	6 (3-15)	7 (3-17)	10 (4-26)	11 (3-24)

All values in median (RR); RR-Reference ranges (2.5- 97.5 percentile).

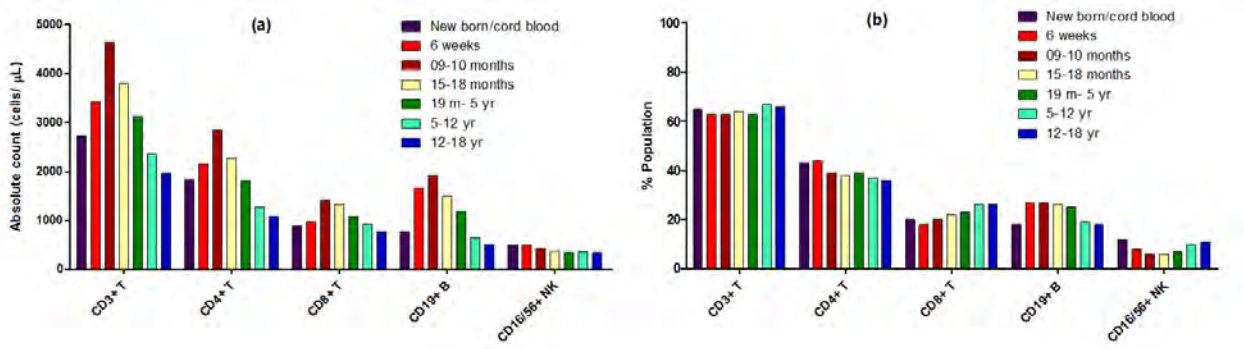

Fig. 1 The median values of each lymphocyte subsets in study groups. (a) Median values of absoplute counts, and (b) proportion of CD3+ CD4+, CD8+, CD19+ and CD16-56+ NK cells in all seven study groups.

significantly different in all lymphocyte subsets where as for other groups the values were different for eg in CD3, CD8 and CD19 percentages and absolute CD4 counts. The NK cell values were generally similar showing difference only in group I in case of North and South regions and in group VII in East and South regions (**Suppl. Table I**). Due to insufficient number of study participants from West region in groups II, III and IV, and from South region in groups II, the comparisons could not be made.

DISCUSSION

In this multi-centric study, we determined the reference ranges for different lymphocyte subsets in Indian pediatric population. This study represents the largest dataset for the relative frequencies of major lymphocyte subsets in healthy Indian children at various age groups from birth till 18 years of age. Unlike CD4+ and CD8+ T cells reference ranges, limited information is available on other lymphocyte subsets like CD3+T cells, CD19+ B cells and CD16/56+ NK cells which have important immune functions.

Our observations confirmed the previous findings that the lymphocyte compartments of normal healthy children differ considerably in various age groups [8-11]. The absolute T cell and B cell count increased during the first few months till 9-10 months and decreased gradually from 15-18 months onwards till 12-18 years. Whereas the relative percentages of T cells i.e. CD3+, CD8+ and CD4+ cells remained more or less similar in all age groups. Similar findings have been reported in African and Caucasian populations previously [10], and from children from southern India [3].

We found that the reference ranges in our cohort differ from pediatric population from other regions like Europe [6,8,12], Africa [13,14], and North America [11,15]. Among the newborns, the absolute cell counts for CD3+, CD4+, CD8+, CD19+ B cells were lower than the cohort from Italy [8] but higher than the African cohort from Cameroon [14]. These differences could be due to the differences in the total lymphocyte percentages and absolute counts, which were not measured in both the studies. Similarly the percentages of CD3+, CD4+ cells of the newborns were lower than Italian children [8] but higher than Cameroon [14], while the percentages of CD8+ T and CD19+ B cells were higher in our newborn group than the newborns from Italy and Cameroon. The number of samples tested from the newborn group might be the reason for such differences. The Italian study used 16 samples from the 0-3 month group whereas the Cameroon study used 38 cord blood samples. In other age groups of children in our study, the absolute CD4 counts were higher than seen in children from Europe, Africa and USA; however in children from 6 years to 18 years, it was comparable to children from Uganda [16]. Except in newborns, children in all age groups had a higher CD8 cell counts when compared with the children from Europe (Italy), Africa (Tanzania, Uganda, Cameroon) and USA [8,11,14,16]. The CD19+ B cell and NK cell counts were higher than the counts observed from Italian population [8] and largely comparable with the pediatric population from Cameroon [14]. Rathore, et al. [17] compared the different immune cells subsets in newborns from United States and India and found that Indian newborns had higher NK and CD4+ T cells, while lower subsets of total T cells, than the American cohort. In comparison with these values, the data from the present study showed lower CD4 counts whereas the CD8+ T cells, B cells, NK cell counts were in similar ranges. Similar to the absolute count, the percentages of different immune cells also varied in our pediatric cohorts in comparison to that of Europe, Africa and USA [8,14,16,18]. These data collectively indicate that each immune cell subset in different age groups of children varies with the ethnicity and is influenced by the geographical region. The lymphocyte subsets are known to vary with the time of collection, use of different

WHAT THIS STUDY ADDS?

- Reference ranges are provided for different lymphocyte subsets in pediatric population from different geographical locations in India.

equipments, procedure for estimation, and the time between the collection and testing [8,9]. Hence, to minimize the variation within the laboratories, proper quality control measures were taken such as use of standard procedure, sample collection in the forenoon hours at all the study sites to avoid diurnal variation, and uniformity of equipment and reagents across the sites. This pediatric cohort did not show significant differences between the sexes, as also observed in other studies [10]; although, reference ranges for the CD4 count and percentages in Indian adults were significantly higher in women [2].

India is a geographically heterogeneous country, hence it was important to assess whether the reference ranges differ in different geographical regions. Our study showed significant differences between the region-wise ranges (mostly East and South) in various parameters in different age groups. These differences might be due to the environmental, genetic or nutritional factors [19-21]. Since this data could not be obtained, these observations need to be confirmed on the larger sample size from the specific age-groups. Moreover, the established ranges could be reconfirmed on a small subset from time to time as described earlier [18]. One of the limitations of our study is insufficient samples available in some regions for children belonging to groups II and III. This could be the due to less number of babies coming for DPT immunization during the study period. It would have been interesting to examine the activation and functional profile of these cells; however, due to the limitation of the parameters that can be tested by the available flow cytometer, it could not be evaluated but could be an important area of future research.

In summary, this study provides reference values for different lymphocyte subsets in Indian children of varying age groups. Age was the only important variable affecting the counts, and sex and geographical distribution did not prove to be significant variables. This data can find application in immune system evaluation of children of Indian origin irrespective of sex, geographical distribution and ethnicity. These age related reference ranges will be helpful to assess the immune defects, and suppression/absence of one or more immune functions in Indian children with primary and secondary immunodeficiencies as well as in autoimmune diseases.

Note: Supplementary material related to this study is available with the online version at *www.indianpediatrics.net*

Ethics clearance: ICMR-NARI Ethics Committee; No. NARI/Age Lymphocyte subsets/10-11/100, dated 22 June, 2010.

Contributors: MT: Study design, data analysis, preparation and review of manuscript; VS: analyzed the data, drafted and reviewed the manuscript; NJ,VR,AD,SS,NS,RM,AS,MC,MM: study design, execution of study, patient information, data analysis, manuscript review (at different sites); SS: data analysis, manuscript preparation and review; AM: study design, data analysis manuscript review; VM: study design, execution of the study and review of the program. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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Supplementary Table I Lymphocyte Subsets in Healthy Indian Children From Different Geographical Regions (*N*=1104)

Region	*Group I:Newborn / cord blood*				*Group II: 6- 32 wk*				*Group III: 9 -10 mo*				*Group IV : 15-18 mo*			
	North (n=51)	*East (n=50)*	*West (n=44)*	*South (n=49)*	*North (n=39)*	*East (n=23)*	*West (n=5)*	*South (n=0)*	*North (n=24)*	*East (n=49)*	*West (n=3)*	*South (n=49)*	*North (n=28)*	*East (n=46)*	*West (n=11)*	*South (n=47)*
CD3+cells Absolute counts	2321	3241.5	2713.5	2676	3001	4326	NA	NA	4148	4805	NA	4442	3728	4143	NA	3484
% CD3+cells	62	66	65.5	67	63	64	NA	NA	64	60	NA	64	59	60.5	NA	69
CD4+cells Absolute counts	1497	2104	1871.5	1744	2081	2770	NA	NA	2498.5	2977	NA	2840	2108	2476	NA	2036
% CD4+cells	36	43	45	44	42	46	NA	NA	44	38	NA	40	38	37.5	NA	38
CD8+cells Absolute counts	789	1048.5	712.5	889	854	1059	NA	NA	1272	1540	NA	1357	1363	1425.5	NA	1250
% CD8+cells	20	22	18	20	17	18	NA	NA	20	21	NA	20	21.5	21.5	NA	25
CD4/CD8 ratio	1.72	1.91	2.53	2.18	2.22	2.52	NA	NA	2.22	1.89	NA	1.96	1.80	1.79	NA	1.52
CD19+cells Absolute counts	797	795	793.5	459	1392	1727	NA	NA	1634	2167	NA	1836	1771	1746.5	NA	891
% CD19+cells	21	17	19.5	13	27	27	NA	NA	27	30	NA	25	30	29.5	NA	19
CD16/56+ cells Absolute counts	426	517.5	413	648	490	453	NA	NA	452	425	NA	407	442	342.5	NA	355
% CD16/56+cells	9	11.5	11	18	8	6	NA	NA	7	7	NA	6	6	6	NA	7

Region	*Group V 19 mo-5 y*				*Group VI 5 y-12 y*				*Group VII 12 y-18 y*			
	North (n=44)	*East (n=54)*	*West (n=49)*	*South (n=50)*	*North (n=55)*	*East (n=44)*	*West (n=52)*	*South (n=59)*	*North (n=40)*	*East (n=21)*	*West (n=38)*	*South (n=50)*
CD3+cells Absolute counts	3129	3380	3141	2869	2350	2539	2273	2264	1870	2051	2128	2024
% CD3+cells	64.5	62	62	66.5	65	68	65	70	65	72	66.5	66
CD4+cells Absolute counts	1797.5	1996	1791	1707	1236	1327	1154	1364	955	1050	1068	1150
% CD4+cells	39	36.5	36	40	34	36.5	36	40	34.5	36	35	37
CD8+cells Absolute counts	1091	1156.5	1109	1045	876	950.5	892	905	675	785	757.5	819.5
% CD8+cells	23	21	21	25	25	25.5	26	27	25	29	25	25.5
CD4/CD8 ratio	1.75	1.87	1.81	1.61	1.42	1.42	1.35	1.48	1.47	1.26	1.34	1.46
CD19+cells Absolute counts	1171	1358	1207	962	667	697.5	623	641	499	322	528	630
% CD19+cells	23	26.5	25	23	19	18.5	19.5	19	18	14	19.5	20
CD16/56+ cells Absolute counts	357	403.5	334	259.5	421	360.5	377	325	324	257	355.5	349.5
% CD16/56+cells	7	7.5	8	7	11	9	10	9	12.5	8	12	11

The table shows the region-wise median values of each parameter and each age group. These values were compared with the respective overall median values (mentioned in table II for the difference. There was a significant difference between the region wise values and overall values (as indicated by the cells filled with gray color) denotes significant difference. The value of $p<0.05$ was considered to be significant. NA indicates 'not applicable' due to insufficient number of children in the group.

RESEARCH PAPER

Patterns and Trends of Childhood Cancer Incidence (0-14 Years) in Delhi, India: 1990-2014

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Objectives: To investigate the patterns and temporal trends of childhood cancer incidence (0-14 years) in Delhi from 1990 to 2014.

Methods: The new childhood cancer cases diagnosed between 1990 and 2014 were extracted from the Delhi population-based cancer registry (PBCR). Joinpoint regression analysis was performed to assess the temporal behaviour of new childhood cancer. The magnitude of temporal trend was assessed by estimated annual percentage changes (EAPCs).

Results: The Delhi PBCR registered 12,637 cases (8484 boys and 4153 girls) during 1990-2014. The overall childhood cancer was twice in boys than girls (5.62% vs. 2.78%). The age-standardised incidence rates (ASIRs) of childhood cancer adjusted to the WHO World standard population distribution (year 2000) was 163 per one million in boys and 92 per one million in girls; median age at diagnosis being 6 and 7 years, respectively. Five-top childhood cancer sites was leukaemia, lymphoma, central nervous system (CNS), bone and retinoblastoma. A decreasing linear trend in proportion of new childhood cancer cases to total all age-group cancer was observed in both sexes during this period. The percentage increase in childhood cancer is similar in both sexes from 1990-94 to 2010-14 (97% vs. 93%). Increasing trend in ASIRs of childhood cancer was observed. **Conclusion**: The new childhood cancer cases observed increasing trend during 1990 to 2014. Boys had nearly double the number of childhood cancer cases than girls while population ratio of boys and girls during the same period was 1.14:1.

Keywords: *Age-standardized incidence rate, Annual percentage change, Epidemiology*.

Childhood cancer incidence is increasing worldwide; developing countries have higher incidence and mortality as compared to developed countries [1]. Globally top-five childhood cancer sites are leukaemia, lymphoma, central nervous system (CNS), kidney and liver, with boys showing a higher proportion of cancer than girls [2,3]. Age standard incidence rate (ASIRs) in India for childhood cancer were 91 per one million and 65 per one million in boys and girls respectively [3].

According to a recent report based on 28 population based cancer registries (PBCRs), the proportion of new childhood cancer to total cancer varied from 0.8% - 4.7% in boys and 0.5% - 2.6% in girls during 2012-2016. This may be due to variation in environmental exposures or biological susceptibility within Indian regions [4]. Delhi showed the highest proportion of childhood cancer for both boys (4.7%) and girls (2.6%) compared to other region of the country. Delhi observed highest ASIRs among boys (203.1 per one million) as well as among girls (125.3 per one million) based on 2012-2014 data. The various studies have been conducted to study the temporal trend of different cancer sites other than childhood cancer in Delhi [5,6]. We examined the patterns and temporal trend of childhood cancer in urban Delhi from 1990-2014.

Editorial Commentary: Pages 415-16

METHODS

The study is based on data extracted from Delhi PBCR, one of the oldest cancer registries of India established in 1986, for all new childhood cancer cases diagnosed between 1990 and 2014. The new cases of top five childhood cancers – Leukaemia (C91-C95), Lymphoma (C81-C85, C96), Central Nervous System (C70-C72), Bone (C40-C41), and Retinoblastoma (C69) were summarized according to gender into five 5-year period (1990-1994, 1995-1999, 2000-04, 2005-2010, 2010-2014). This registry fulfilled the IARC data quality standards and the data was published in cancer incidence in five continents volumes IX and volume X [7,8]. The

international classification of disease for oncology (ICD-O) code 9th revision was used for the period 1988-2000 and 10th revision was utilized for the period 2001-2014.

According to the 2011 census, the total population of Delhi was 1,67,53,235 with 97.5% of people living in urban areas. Out of these, 0-14 years population was 45,65,319 and about 97.1% of 0-14 them live in urban areas of Delhi.

The target population for 0-14 years used in determining the incidence rates of respective years was estimated from the 1991, 2001, 2011 census reports of India using difference distribution method [9,10]. These estimates were not adjusted for immigration and fertility changes over the period due to non-availability of adequate data. ASIRs were calculated by the direct method using WHO World standard population distribution, year 2000 [11].

Joinpoint regression model using joinpoint regression programme [12] was applied to assess the magnitude of time trends on the ASIRs of top five childhood cancers and total childhood cancer during 1990-2014 according to gender. ASIR was determined using the formula: Number of new childhood cancer cases in a specific age-group during a period*1000,000/Estimated population of a specific age group during the period.

Simple linear regression was performed to assess the trend of relative proportion of new childhood cancer cases to all age-group cancers. The percentage increase in top-five new childhood cancer cases was calculated by: (childhood cancer cases during 2010-2014 minus childhood cancer cases during 1990-1994) *100 / Childhood cancer cases 1990-1994. The median age was determined using the median formula for group data on combined 25-years data.

The estimated annual percentage changes (EAPCs) in cancer incidence rates was calculated by fitting a jointpoint regression model, assuming a constant rate of change in the logarithm of the annual ASIRs in each segment. The significance of EAPC was tested using asymptotic t-test and considered significant at 5% if 95% Confidence interval (CI) of EAPC does not include zero. The connecting points of the jointpont segment was treated as break. For 25-data points, joinpoint software recommended a maximum 4 joinpoints. This started with a minimum zero *i.e* straight line. Monte Carlo permutation test with 4499 randomly permuted data sets was applied to test the additional requirement of joinpoint, the obtained *P*-value was adjusted according to Bonferroni correction due to multiple comparisons [13]. ASIRs show a rising trend when the point estimate and lower limit of 95% CI are >0. In contrast, a decreasing trend can be seen when the point estimate and upper 95% CI are <0. Else, the ASIRs are deemed to be stable over the time period. The multiple comparisons, asymptotic t-test and Monte Carlo permutation test are part of joinpoint software [12].

RESULTS

In 1990-2014, Delhi PBCR registered 12,637 new childhood cancer cases (8484 boys and 4153 girls). The proportion of childhood cancer was 4.2% relative to total cancers of all-age groups. Childhood cancer amongst boys relative to all age-groups cancer was almost double than in girls (5.62% vs. 2.78%) while the ratio of population of boys and girls was 1.14 to 1 during this period. The combined five top childhood cancer during 1990-2014 were leukemia [C91-C95], lymphoma [C81-C85], CNS tumors [C70-C72], bone tumors [C40-C41] and retinoblastoma [C69] (**Table I**). The boys observed a similar sequence of top-five childhood cancer during the recent three five-year intervals. While, girls observed different sequence, (leukemia, CNS tumors lymphoma, bone tumors and retinoblastoma). Top five childhood cancers contributed nearly 80% among boys and 70% among girls respectively. Leukemia was a prominent cancer in both gender and contributed 36.1% (approximate one-third) of total childhood cancer cases (**Table I**). The proportion of childhood cancer to total cancer of all age groups revealed a significant linear decreasing trend in both boys and girls. This percentage of childhood cancer decreased from 6.6% to 4.7% in boys and 3.6% to 2.6% in girls respectively from 1990 to 2014 (**Suppl. Fig. 1**). Median age at diagnosis of childhood cancer for boys was 6 years and for girls 7 years while in combined it was 6 years.

Over the period of 1990-94 to 2010-14, the percentage increase in new childhood cancer cases was almost similar for both the gender (97% vs. 93%). The

Table I Proportion of Childhood Cancer Cases During 1990-2014 in Delhi

Type of Childhood Cancer	*Boys n=8484*	*Girls n=4153*	*Total n=12637*
Leukemia	3127 (36.9)	1438 (34.6)	4565 (36.1)
Lymphoma	1517 (17.9)	376 (9.0)	1893 (15.0)
Central nervous system	997 (11.8)	498 (12.0)	1495 (11.8)
Bone	570 (6.7)	351 (8.4)	921 (7.3)
Retinoblastoma	434 (5.1)	232 (5.6)	666 (5.3)
Other types[a]	1839 (21.7)	1258(30.3)	3097 (24.5)

All values in no. (%). [a]Other types of childhood cancer.

girls observed higher percentage change in leukemia, retinoblastoma, and other cancers sites, while lymphoma, and CNS tumors was more in boys during 2010-14 relative to 1990-94. The percentage increase of bone cancer was almost similar in both genders (**Fig. 1**).

The age specific (0-4,5-9,10-14, and 0-14 year) distribution of top-five childhood cancer over the 25-year period (1990 to 2014) was similar in boys and girls except for lymphoma which was more frequent in boys. Retinoblastoma was more common in children <10 years. Although girls had higher counts under five-year compared to the remaining two five-year age groups albeit in boys under 5-year and 5-9 years age-group had similar number of cases. The age-specific incidence rate per one million of childhood cancer was higher in 0-4 years as compared to two subsequent 5-year intervals among both gender (**Suppl. Fig. 2**).

The age-period graphs for top-five cancers as well as total childhood cancer according to gender are included as **Suppl. Fig. 3** and **4**. In boys, total childhood cancer and leukemia had an upward incidence pattern in recent two periods than the remaining three periods in nearly all the three childhood age groups. In girls, total childhood cancer, leukemia and bone cancers showed increasing trend in all childhood age groups from 2005 to 2014 **(Suppl. Fig. 2** and **3**). Lymphoma was more frequent in 5-9 years age-group, bone in 10-14 years age-group, and retinoblastoma in 0-4 year age groups among the boys, similar pattern of these sites was observed among the girls.

The ASIRs of childhood cancer was 163.2 per one million (95% CI:159.8-166.7) in boys and 91.6 per one million (95% CI:88.8-94.4) in girls. (**Table II**). The trend analysis showed a significant increase in ASIRs of childhood cancer, EAPC 1.53% (95% CI: 0.87 to 2.13) in boys and observed one break among girls, the trend remained stable between 1990-2005, but increased more rapidly with 6.0% per year these after (**Fig. 2, Table II**).

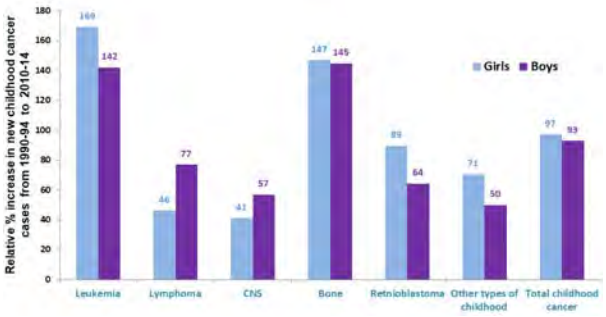

Fig. 1 Comparison of relative percentage change in top-five childhood incidence cases from1990-94 to 2010-2014 between boys and girls.

For pooled data, no change in ASIRs trend was seen till 2004, but a significant rise was observed with EAPC 4.0% per year in recent decade (2004-2014) [data not shown].

The childhood cancer trends varied according to cancer site as well as by gender. In boys, upward trends were observed for lymphoma (EAPCs= 1.25% per year), and for bone tumors (EAPC 2.51% per year). However, leukemia and CNS tumors observed no change between 1990 and 1999, but found a positive significant trend with EAPC of 4.6% per year and 3.1% per year from 1999 to 2014. The girls showed a different trend than boys; one break was observed in CNS tumors with a 2.6% decrease in EAPC from 1990 to 2007 and remained stable since then (**Table II**). Leukemia also observed one break with a stable incidence trend between 1990-2004 and a sharp upward trend in recent decade with EAPC of 7.0% per year. Bone tumors had a significantly rising with EAPC of 3.2% per year during 1990-2014. Lymphoma, retinoblastoma and other childhood cancers sites depicted a consistent flattening trend over the period.

DISCUSSION

The percentage of new childhood cancer cases to total cancer cases were significantly decreasing in both genders. This may due be to the falling trend of fertility rate and growing trend of expectancy of age in Delhi as well as in India [14]. The total fertility rate decreased from 4.83 in 1980 to 2.3 in 2015 as per United Nation websites [15]. In Indian urban areas, life expectancy at birth increased from 65.4 during 1990-94 to 71.5 during 2010-2014. The life expectancy at birth in Delhi during 2010-14 was 73.5 [16].

ASIRs of childhood cancer showed an increasing trend during 1990-2014 in boys and in girls during 2005-2014.

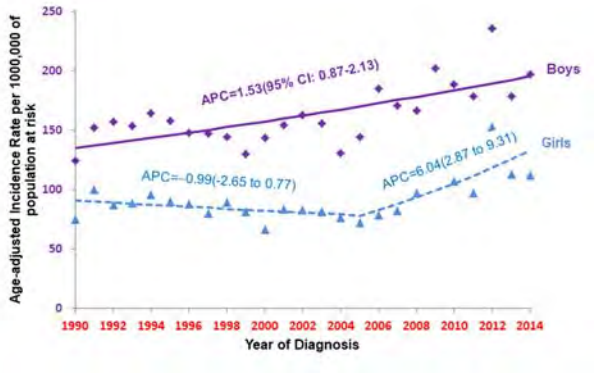

Fig. 2 Trend of age-standardized (world population, per one million) incidence rates for childhood cancer in Delhi urban area between 1990 to 2014 (trend modelled using joinpoint regression).

Table II Age-standardized Rates and Time Trend of Five Top-childhood Cancer in Delhi Urban Area by Sex: 1990-2014

Cancer Type	*Age-standardized Incidence rate/ million population/year*[b]						*Estimated Annual Percentage Change [EAPC(95%CI)]*	
	1990-94	*1995-99*	*2000-04*	*2005-09*	*2010-14*	*1990-2014 (95% CI)*[b]	*Trend-1*	*Trend-2*
Boys								
Leukemia [C91-C95]	50.8	46.0	50.1	65.7	82.1	60.2 (58.0 to 62.3)	-1.27 (-5.06 to 2.67)[a] [1990 to 1999]	4.58 (3.16 to 6.01) [b] [1999-2014]
Lymphoma [C81-C85,C96]	27.5	23.2	27.4	31.4	31.9	28.5 (27.1 to 30.0	1.25 (-0.006 to 2.3) [a]	
CNS [C70-C72]	20.3	16.6	16.1	21.2	21.2	19.1 (17.9 to 20.3)	-3.20 (-8.1 to 1.90)[a] [1990-2000]	3.10 (6.5 to 5.6)[c] [2000-2014]
Bone [C40-C41]	8.3	8.7	10.1	12.0	12.8	10.6(9.8 to 11.5)	2.51 (0.88 to 4.16)[c]	
Retinoblastoma [C69]	8.5	9.1	7.5	9.0	9.9	8.8(8.0 to 9.64)	1.09 (-0.73 to 2.94) [a]	
Other CC	35.2	40.3	36.5	32.8	35.8	36.1 (34.41 to 37.7)	0.41 (-0.53 to 1.36)[a]	
Total	150.5	143.9	147.8	172.2	193.6	163.2 (159.8 to 166.7)	1.53 (0.87 to 2.13)[b]	
Girls								
Leukemia [C91-C95]	23.8	29.1	23.23	34.4	45.0	32.1 (30.1 to 33.4)	0.37 (-3.06 to 3.92) [a] [1990-2004]	7.06 (2.7 to 11.6)[c][2004-2014]
Lymphoma [C81-C85,C96]	9.1	7.4	7.1	8.3	9.2	8.0 (7.4 to 9.01)	0.41 (-1.29 to 2.14) [a]	
CNS [C70-C72]	13.3	10.1	10.2	8.3	13.0	10.9 (9.9 to 11.9)	-2.6(-4.7 to -0.44)[c][1990-2008]	10.3 (-0.27 to 21.96); [a] [2008-2014]
Bone [C40-C41]	6.8	5.3	6.2	7.8	10.9	7.5 (6.7 to 8.3)	3.2 (1.61 to 4.79)[b]	
Retinoblastoma [C69]	5.2	5.0	3.4	5.3	7.5	5.3 (4.6 to 6.0)	1.92 (-0.76 to 4.80) [a]	
Other CC	30.2	28.4	27.5	24.1	30.0	28.0 (26.4 to 29.5)	-0.16 (-1.32 to 1.02)[a]	
Total	88.3	85.3	77.7	88.1	115.6	91.6 (88.8 to 94.4)	-0.99 (-2.65 to 0.71) [a] [1990-2005]	6.04 (2.87 to 9.31) [d] [2005-2014]

[b] age-standardized to the WHO World standard population, year 2000[11] : CNS = Central Nervous System. [a]P>0.05; [b]P<0.001; [c]P<0.05; [d]P=0.001.

Our results of rising trend of new childhood cancer cases was akin to Canada [17], Australia, and Taiwan [18,19] **(Suppl. Table I)**. To the best of our knowledge, no Indian study has assessed the time trend analysis of new childhood cancer cases, albeit one study reported rising trend in childhood cancer among the Indian PBCRs comparing three time periods [20]. Leukemia contributed to 36.1% of total childhood cancer cases as also seen in Canada [32.4%; 1992-2010], Australia [32.4%; 1983-2006], Taiwan [33.9%; 1996-2010], Estonia [31.1%;1970-2016], Thailand [36.1%: 1990-2011]. [17-19,21-22]. Globally, the next two commonest childhood cancers are CNS tumors with range 13.9%-22.7%, followed by lymphoma with range 10%-11.2%, albeit in urban Delhi lymphoma and CNS tumors account for with a percentage of 15.0% and 11.0% of all the childhood cancer cases respectively [17-20]. However, in Chennai lymphoma and CNS tumors account for 20% and 11% of all childhood cases respectively [23].

ASIR for childhood cancer in Delhi was 129.8 per one million [95% CI: 127.6-132.1] from 1990-2014 which is closer to the Taiwan (125.0 per one million from 1996-2010) [19] and lower than United States (172.8 per one million between 2007 and 2011) [24] and Australia (157.5 per one million from 1997-2006) [18].

The etiology of childhood cancer is still limited, some of the factors like environmental exposure, genetic, parental smoking, higher birth weight, and high maternal age are associated with most childhood cancers [25,26]. A large US case-control study reported an increase of 8% in overall childhood cancer risk for each 5-year increase in maternal age [27]. Likewise, an increasing trend in median marriage age was observed in India [28]. The main reasons for increasing median age are rise in female workforce and higher education enrolment of females especially in urban India [28]. Indirectly, the median age of first-time mother showed an increasing trend but exact magnitude cannot be estimated.

The change in trends could also be influenced due to shift in coding or registration practices and improvement in diagnostic advancement during the period. The immigrants might also contribute to increase in new childhood cancer cases. Immigration in Delhi steadily increased from 6.34 lakhs during 1961-1971 to 22.2 lakhs during 2001-2011 [29]. However, effect of this increase on

WHAT THIS STUDY ADD?

- There is approximate 100% increase in incidence of reported childhood cancer cases from 1990-94 to 2010-2014 in urban Delhi.
- Median age at diagnosis of childhood cancer in Delhi is 6 years.

childhood cancer cannot be ascertained due to non-availability of data.

The main strength of Delhi PBCR is almost complete coverage and collection high-quality data. The Delhi PBCR data is also included in the International Agency for Research on Cancer scientific publications [7,8]. Although Delhi PBCR collects the data of only those patients who have been residing for at least one year in Delhi but veracity cannot be confirmed. The projected population of each year to calculate the ASIRs does not adjust for immigration and fertility changes over the years which may over-estimate the incidence rate. The subgroups of leukemia and lymphoma trend could not be observed due to a small number of cases. Age-period-cohort (APC) model that assess the effect of age, effect of period, and effect of cohort on the incidence, was not performed in the present study. The join point analysis developed by National cancer Institute, USA, is a robust method to assess the trend analysis especially for cancer incidence and mortality data [12,13].

The trend of overall new childhood cancer cases showed a significant increase especially in the latest decade in Delhi for boys and girls. The contribution of childhood cancer to total cases showed a decreasing trend during this period. Compared to girls, boys had doubled childhood cancer cases during 1990-2014. Leukemia is the most common cancer site and contributed to one-third of total childhood cancers during the 25-year period. The trend and knowledge of present status of childhood cancer helps the public health policy makers as a baseline for future planning and allocation resources.

Note: Supplementary material related to this study is available with the online version at *www.indianpediatrics.net*

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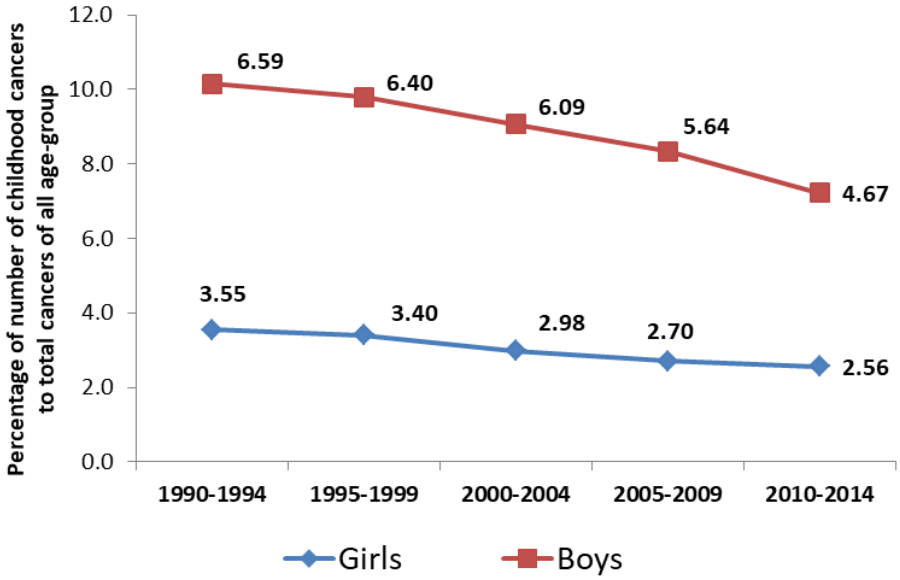
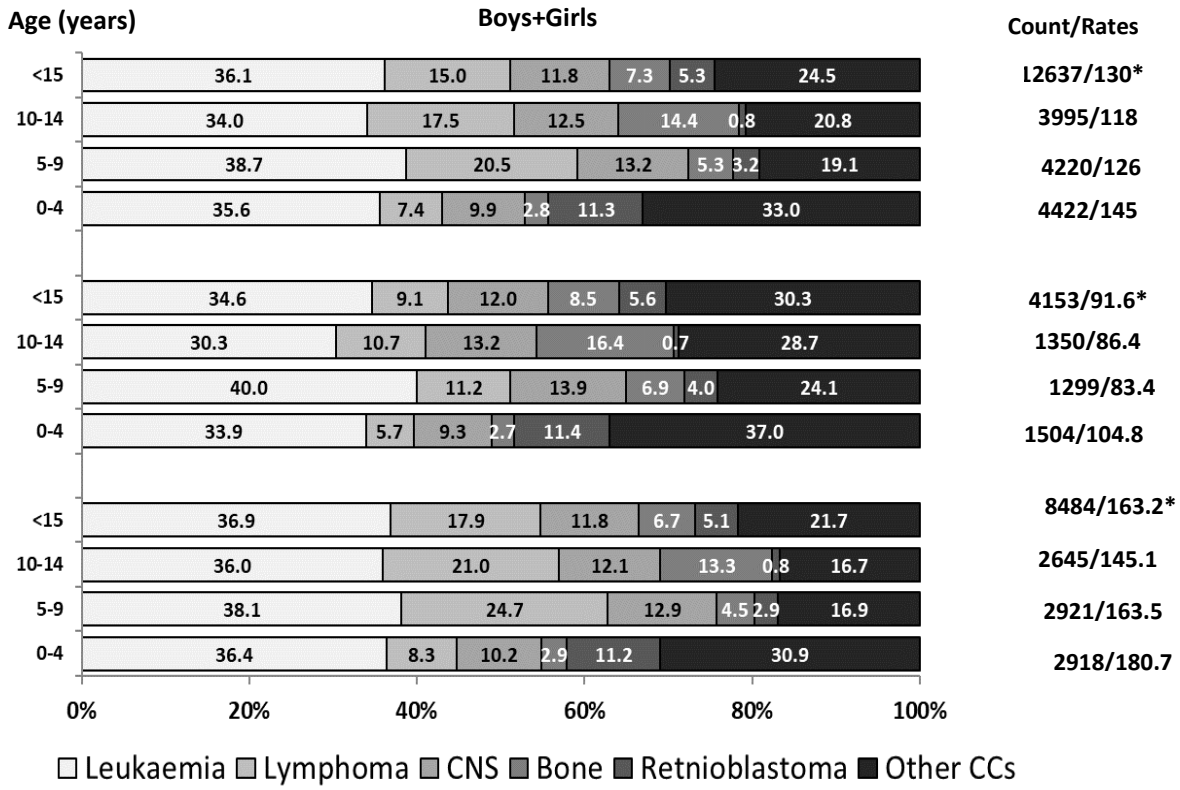
Supplementary Table I Comparison of Incidence Trend and Age-standardized Incidence Rate of Childhood Cancer Among Various Countries

Country Name	*Study period*	**APC(95% CI)*		*Age-standardized incidence rate*
		Trend-1	*Trend-2*	
Canada [17]	1992-2010	0.45 (0.08-0.81)	-	157.9[b]
Australia [18]	1983-2006	1.7 (0.9 to 2.5) 1983-1994	-0.1(-0.7 to 0.06) 1995-2006	157.5 (153.6-161.5)[a]
Taiwan [19]	1996-2010	1.21 (0.6 to 1.7)	-	125.0 (122.3-127.7)[a]
Estonia [21]	1995-2016	0.5 (0.1-0.9)	-	138.1[a]
Thailand [22]	1990-2011	1.2 (0.8-1.7)	-	98.5[c]
Present Study (Delhi, India)	1990-2014	-0.22 (-1.75 to 2.21) 1990-2004	4.05 (1.85-6.29) 2005-2014	129 (127.6-132.4)[a]

** Annual percentage change in incidence rate using Joinpoint regression analysis Incidence rates were age-standardised using [a]WHO world standard population distribution, year 2000 [b]Canadian population distribution, year 2011, [c]Segi's et als. World standard population estimates, year 1960*

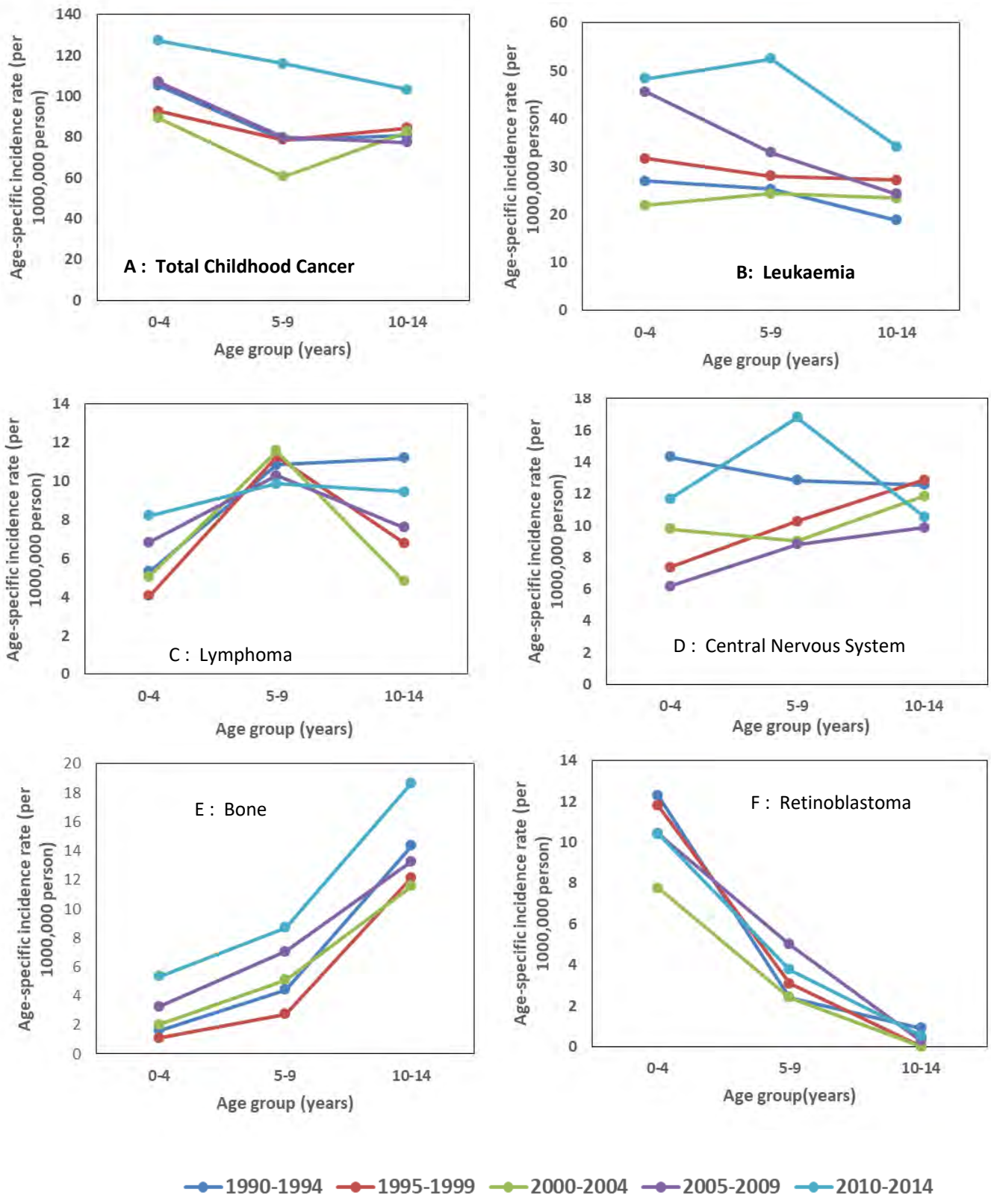


Supplementary Fig. 1 Trend of percentage of new childhood cancer cases to total all-age group cancer cases. (Girls: slope =-0.05; *P*=0.002 and Boysslope=-0.092 *P*=0.013)

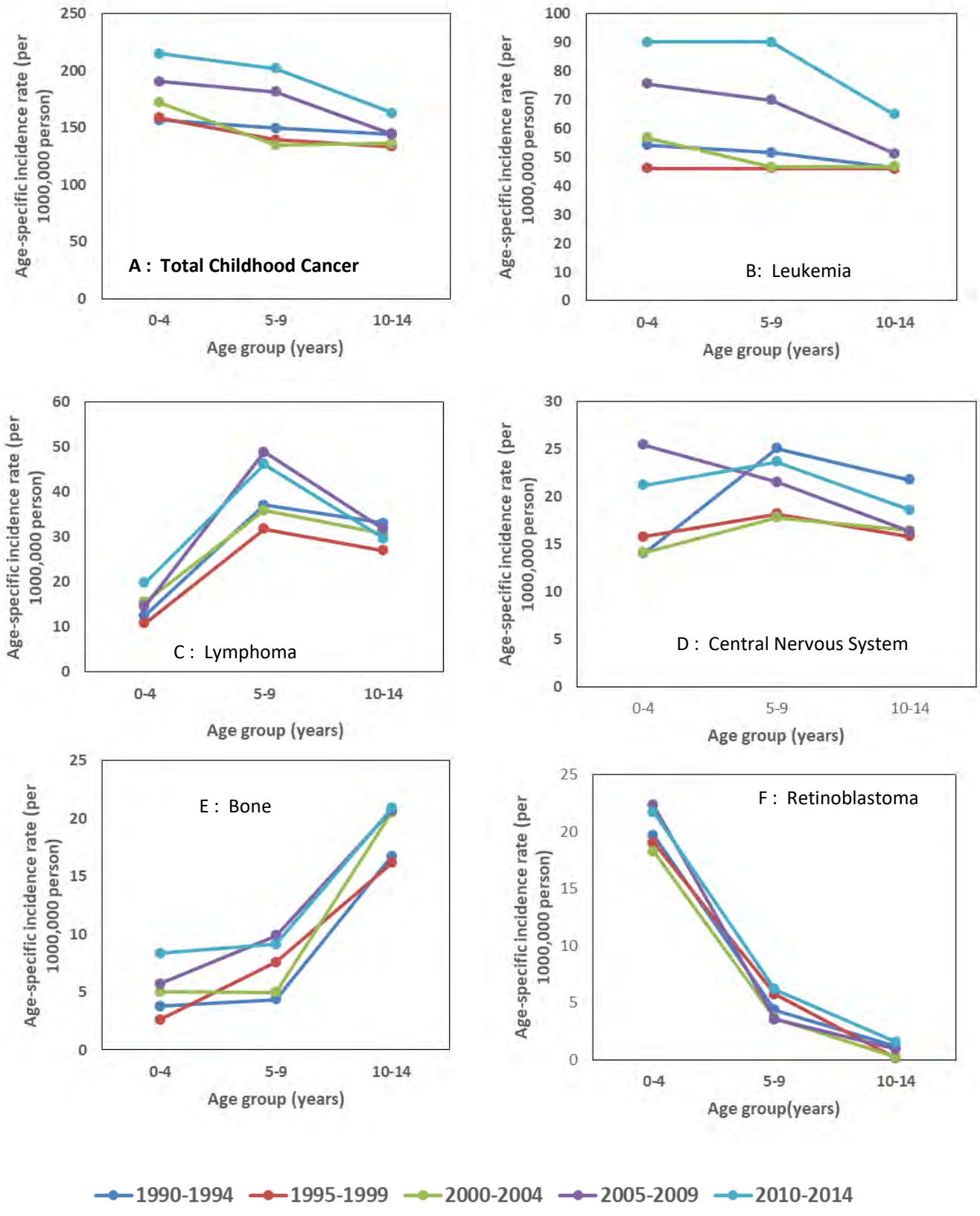


**The rates were standardized according to WHO World population distribution, year 2000 using direct method [11]*

Supplementary Fig. 2 Childhood cancer (0-14 years) distribution by gender and age-groups, Delhi, 1990-2014.



Supplementary Fig. 3 Age-period diagram of age-specific incidence rate in girls during 1990-2014 in Delhi urban, India



Supplementary Fig. 4 Age-period diagram of age-specific incidence rate in boys during 1990-2014 in Delhi urban, India.

RESEARCH PAPER

Obesity and Sarcopenia in Survivors of Childhood Acute Lymphoblastic Leukemia

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Objective: To describe the prevalence of obesity and sarcopenia among survivors of childhood acute lymphoblastic leukemia (ALL) using DEXA scan, and study associated risk factors. **Methods:** This case control study was conducted between July, 2013 and June, 2014 at a tertiary care cancer centre in India. Study participants included 65 survivors of childhood ALL who were <18 years of age at diagnosis, treated between years 1996 and 2008, and were at least two years since completion of therapy. The controls included 50 matched siblings. Dual energy *X*-ray absorptiometry (DEXA) was used to study the body composition (body fat percentage, BF% and lean body mass, LBM) of the participants and controls. McCarthy's body fat reference data were applied and logistic regression analysis was used to study various risk factors. **Results:** At a median (range) follow-up of 5 (7.2-17.2) years, BF% (DEXA) identified a significantly higher prevalence of obesity of 21.5% (14/65) and sarcopenic obesity (14%) among survivors as compared to the controls (0/50, *P*<0.001), while the prevalence of sarcopenia as detected by LBM was similar at 60% (39/65) and 56% (28/50), respectively. On multivariate analysis, age at evaluation, high-risk disease and cranial irradiation were independently associated with high likelihood of obesity, while none of the factors predicted sarcopenia. **Conclusion:** High prevalence of obesity and sarcopenic obesity were observed among survivors of childhood ALL.

Keywords: *Body composition, Body fat, DEXA, Lean body mass, Metabolic syndrome.*

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Obesity is recognized as a common chronic health problem in childhood cancer survivors, and cardiovascular disease has been shown to occur at an earlier age in survivors of childhood cancer [1-4]. As acute lymphoblastic leukemia (ALL) is the commonest childhood cancer, we aimed to study the prevalence of obesity and sarcopenia among survivors of childhood ALL using DEXA scan, as compared to sibling controls, and evaluate association with risk factors.

METHODS

This study was conducted between July, 2013 and June, 2014 at the pediatric hemato-oncology department of our comprehensive cancer center. The study participants (cases) included survivors of childhood ALL who were less than 18 years of age at diagnosis, treated between 1996 and 2008, in first complete remission, and were at least two years after completion of therapy. Of the total 207 ALL patients that were treated, 122 were eligible for the study and we were able to contact and obtain consent from 65 children and young adult survivors (52 males). The treatment protocol was based on a Berlin Frankfurt Munster (BFM) backbone that included a four-drug induction and prednisolone as the glucocorticoid [5]. Their results were compared with 50 healthy sibling controls that were matched for age (±1 year) and sex. The controls were determined to be healthy and eligible for the study if there was no evidence of any medical illness on detailed history and physical examination. The study was approved by the institutional ethics committee and conducted after obtaining informed consent from the study participant or parents (in case of minor).

Details of method of measuring anthropometric indices (weight, height, BMI) and physical activity quotient (PAQ) have been previously published [5]. Three-compartment body composition was assessed using DEXA and Hologic Explorer (S/N 91531) software version 13.3:3. The DEXA machine directly generates absolute values as well as *Z* scores for bone mineral content, and lean body mass (LBM) measures, as the National Health and Nutrition Examination Survey (NHANES) reference data is integrated into the software.

Study participants' body mass index (BMI) status was determined by using WHO BMI growth charts and

categorized as normal weight (BMI >5th to <85th percentile), overweight (>85th to <95th percentile), or obese (BMI ≥95th percentile) [6,7]. High adiposity was defined as body fat percentage (BF%) levels higher than the 85th percentile of McCarthy BF% reference data (NHANES) for each age-sex group (overweight ≥85th percentile and obesity ≥95th percentile) [8,9]. Sarcopenia was defined as LBM <5th percentile of reference data (NHANES). Sarcopenic obesity was defined as participants fulfilling criteria for sarcopenia and obesity by BF% and LBM [8]. Body fat mass index (BFMI) and lean body mass index (LBMI) were calculated from DEXA-measured body-composition data as BF or LBM in kg per m^2 height.

Statistical analyses: The body compositions of two groups such as weight, height, BMI, BF % etc. were compared using independent *t* test and chi-square test. Analysis of variance (ANOVA) was used to compare delta BMI change among thin, average and overweight patients at diagnosis. Univariate and multivariate logistic regressions were used to study the influence of various demographic and disease-related factors for obesity and sarcopenia among the survivors. The results were interpreted using odds ratio (OR) and 95% confidence interval (CI). The Statistical Package for Social Sciences for Window's software (IBM SPSS Statistics version 23.0) was used for all analyses. Significance was set at $P<0.05$.

RESULTS

At the time of evaluation, the 65 study participants [median (range) age, 15 (7.7-27.5) years] were median (range) 4.3 (2-14.8) years from treatment completion, and had a median (range) follow-up of 7.2 (5-17.2) years.

The values for body composition for the survivors and the control groups are provided in **Table I**. The mean (SD) BF% was significantly higher among ALL survivors as compared to the controls [35.2 (7.4) *vs* 30.2 (8.0); $P=0.001$], and a similar trend was observed when analyzed by gender. Using BF%, obesity was observed among 21.5% (14/65) of study participants and none of the controls ($P=0.00$) and overweight among 55% (36/65) and 48% (24/50) of cases and controls respectively ($P=0.7$). On the other hand BMI under-estimated obesity (6%, 4/65, $P=0.02$) and overweight (26%, 17/65, $P=0.01$) among the study participants. It also detected fewer control participants to be overweight (6%, 3/50, $P=0.01$).

We further looked at WHO BMI *z*-scores at diagnosis and noted that 10/65 (15%) were underweight/thin, 9/65 (14%) were overweight, and none was obese. While at evaluation, these proportions changed to 26.1% ($n=17$) overweight and 6.1% ($n=4$) obese, respectively. No participant was underweight/thin. Delta BMI change was calculated from baseline BMI percentile, which revealed that the highest mean (SD) delta change occurred among thin [2067(882)] followed by normal [273(50)] and overweight [45(30)] patients, ($P<0.001$).

The absolute values for LBM and LBMI for the study participants' were lower than the controls but this was not statistically significant ($P=0.38$, 0.68) (**Table I**). The prevalence of sarcopenia was similar in both the groups (39/65, 60%; 28/50, 56%), respectively ($P=0.10$). However, the female survivors (11/13, 85%) were more sarcopenic compared to their male counterparts (28/52, 54%) ($P=0.05$). Sarcopenic obesity was observed among 14% (9/65) of study participants and none of the controls ($P=0.00$). Of these 9 patients, 1 was thin and none were overweight or obese based on baseline BMI, this difference was not statistically significant ($P=0.29$).

Distribution of demographic, disease and treatment exposure among participants who were obese or sarcopenic is displayed in **Table II**. The study participants had a 28 times higher odds of being obese compared to the controls [OR (95% CI) 28 (1.7 to 490); $P=0.002$] . On univariate logistic regression analysis female gender, age at diagnosis less than 10 years, T immunophenotype, high NCI risk, receiving cranial irradiation, more than 5 years since therapy, younger age at evaluation, being sarcopenic, and PAQ>2 were significantly associated with obesity. On multi-variate analysis, only female gender [OR (95% CI) 7.3 (1.1-50.1); $P=0.04$], high NCI risk category [OR (95% CI) 6.7 (1.1-43.5); $P=0.04$], cranial

Table I Body Composition by DEXA Scan and Anthropometry of Survivors of Childhood Acute Lymphoblastic Leukemia and Sibling Controls ($N=115$)

Characteristics	*ALL survivors (n=65)*	*Controls (n=50)*	*P value*
Weight, kg	50.9 (16)	48.5 (16.3)	0.42
Height, cm	154.6 (15.3)	156 (14.9)	0.61
BMI, kg/cm^2	20.8 (3.7)	19.3 (3.8)	0.03
Body fat, %	35.2 (7.4)	30.2 (8.0)	<0.001
BFMI	7.26 (2.4)	5.8 (2.3)	<0.001
Lean body mass	30355.9 (9886.1)	32092.7 (10644.6)	0.38
LBMI	12.3 (2.1)	12.5 (2.5)	0.68
Trunk/Leg fat %	0.9 (0.6)	0.8 (0.5)	1.0
Trunk lean mass	14861 (5000.9)	15835.93 (5509.8)	0.33

All values in mean (SD). DEXA: Dual energy X-ray absorptiometry; ALL: Acute lymphoblastic leukemia; BMI: Body mass index at evaluation; BF%: Body fat percentage; BFMI: Body fat mass index; LBMI: Lean body mass index.

Table II Demographic and Disease Characteristics of Obese and Sarcopenic Survivors of Childhood Acute Lymphoblastic Leukemia (All)

Characteristic	*Survivors (n=65)*	*Obese (n=14)*	*Sarcopenic (n= 39)*
Male	52 (80)	6 (42.9)	29 (74.4)
Age at diagnosis <10 y	19 (29.2)	14 (100)	26 (66.7)
T cell immunophenotype	14 (21.5)	4 (28.6)	8 (20.5)
High NCI risk	30 (46.2)	5 (35.7)	19 (48.7)
Cranial radiation	31 (47.7)	4 (28.6)	19 (48.7)
>5 y after therapy	29 (44.6)	4 (28.6)	19 (48.7)
Age at evaluation <10 y	10 (15.4)	6 (42.9)	7 (17.9)
High BMI	21 (32.3)	14 (100)	1 (2.6)
Sarcopenic LBM	39 (60)	9 (64.3)	39 (100)
Normal PAQ score	21 (32.3)	13 (92.9)	27 (69.2)

All values in no. (%). DEXA: Dual energy X-ray absorptiometry; NCI: National Cancer Institute; BMI: Body mass index at evaluation; LBM: Lean body mass; PAQ: Physical activity quotient.

irradiation [OR (95% CI) 9.9 (1.2-83.3); *P*=0.04], and younger age [OR (95% CI) 10.2 (1.1-91.4); *P*=0.04], had a significant association with obesity.

Among 31 children exposed to cranial RT, doses of 12.6 Gy were administered to 27 of whom one was obese. Four children received cranial RT at doses of 18 Gy and three of them were obese. None of the four obese survivors suffered from endocrinopathies or short stature to account for obesity. None of the baseline characteristics were associated with higher prevalence of sarcopenia.

Table III Multivariate Logistic Regression Analysis of Obese and Sarcopenic Survivors of Childhood Acute Lymphoblastic Leukemia (*N*=65)

Variables	*Odds ratio (95% CI)*	*P value*
Female	7.3 (1.1-50.1)	0.04
Age at diagnosis (<10y)	1.2 (0.2-7.5)	0.85
T Immunophenotype	1.9 (0.1-25.6)	0.63
High NCI risk	6.7 (1.1-43.5)	0.04
Cranial radiation	9.9 (1.2-83.3)	0.04
>5y after therapy	1.6 (0.3-8.6)	0.59
Age at evaluation (<10y)	10.2 (1.1-91.4)	0.04
High BMI	2.1 (0.4-11.5)	0.38
LBMI-Sarcopenic	1.3 (0.3-5.6)	0.72
PAQ >2	8.2 (0.8-83.3)	0.07

NCI: National Cancer Institute; yr: year; BMI: Body mass index at evaluation; LBM: Lean body mass; LBMI: Lean body mass index; PAQ: Physical activity quotient.

DISCUSSION

In the present analysis, using DEXA we observed that more than one-fifth of the survivors of childhood ALL were obese and half of them were overweight at a median follow-up of 7 years. A wide variation in prevalence rates of obesity (18-80%) has been reported among childhood cancer survivors in studies using DEXA scans. The St Jude life time cohort study [10] reported obesity in 63% male and 85% female ALL survivors at a mean follow up of 25 years, while Barr, et al. [11] from Canada reported obesity and overweight rates of 12% and 18% respectively at a median follow-up of 21 years. The range in findings may be attributed to differences in definitions of obesity, treatment protocol and gluco-corticoid doses, duration of follow-up, ethnicity, and social factors as well as the prevalence of obesity in the normal population of the region. Comparison with a control population helped obviate many of these factors. The main advantage of using sibling controls was to avoid confounding biases due to constitutional and environmental factors.

Published studies form India have used weight- and height-based indices and reported lower prevalence rates of obesity (2.5-12%) and overweight (19-20%) [12,13]. In order to be comparable with these data, we adopted the BMI criteria and observed similar rates of obesity (6%) and overweight (24%) among our ALL survivors. Hence, BMI underestimated the prevalence of adiposity as it is unable to identify normal and underweight individuals with high body fat. Blijorg, et al. [14] used total fat percentage as the gold standard, and reported that 42% of male survivors and 65% of female survivors were misclassified as non-obese using BMI [9].

It is noteworthy that the prevalence of obesity and overweight was not influenced by the study participants' nutritional status at diagnosis, since the thin and normally nourished children treated for ALL were equally predisposed. However, we observed that the delta change in BMI was highest amongst those who were under-weight/thin at diagnosis, and they probably require close monitoring during follow-up.

Various investigators have described muscle mass loss during the treatment for ALL and its progression throughout therapy and after treatment completion [15,16]. This is attributed mainly to the degradation and decreased synthesis of myosin heavy chains, and steroid use, which causes increased glycogen and lipid levels in muscle cells. The prevalence of sarcopenia was equally high amongst our control population, which possibly indicates that ethnically, our population has lower muscle mass compared to Western counterparts. We did; however, observe a high prevalence of sarcopenic obesity

WHAT THIS STUDY ADDS?

- Body composition analysis by DEXA scan showed that 21.5% survivors of childhood ALL were obese and 60% had sarcopenia on follow-up.

(14%) among the survivors that was not seen among the controls. This assumes importance in the context of current literature that highlights the combination of the two to be more detrimental and an important contributor to the development of metabolic syndrome [16].

The observed gender difference has previously also been reported [10,11,17]. Hyperleptinemia, which occurs in girls during puberty, has been linked to body fat and has been described as a possible mechanism for obesity. Although many investigators have reported the association of cranial RT with obesity, it remains a controversial point [17]. Disturbances influencing the satiety centre and dysfunction of hypothalamic-pituitary axis have been found to cause obesity as well. However, with modern treatment protocols, wherein smaller doses of radiation are delivered with better techniques, recent papers have revealed no association of cranial radiation with the incidence of obesity in survivors of childhood ALL [18]. The increased incidence of obesity among children with NCI high risk disease status may be attributed to the use of higher doses of glucocorticosteroid therapy, poor physical activity and use of cranial radiation in this subset.

The results of our analysis should be interpreted in light of the small sample size and skewed gender ratio, in addition to the fact that influence of unknown psychosocial factors on the controls could not be completely excluded. However, our use of DEXA to accurately identify adiposity and sarcopenia as well as use of matched controls strengthens our findings.

The findings of the present study highlight the high prevalence of obesity and sarcopenic obesity in our population of survivors of childhood ALL. Since these are believed to be forerunners of cardio-metabolic syndrome our results emphasize the need for early recognition and aggressive preventive strategies. Larger interventional studies may identify strategies that have an impact on reducing obesity in this sub-population of children.

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Contributors: PM: data acquisition, interpretation, drafting the manuscript; GK: study design, data interpretation, reviewing and finalizing manuscript; SJ: data interpretation, reviewing manuscript; AS: data analysis and interpretation, reviewing manuscript.
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CLIPPINGS

Coronavirus infections in the nervous system of children: A scoping review making the case for long-term neurodevelopmental surveillance (*Pediatric Neurol. 2021;117:47-63*)

This scoping review included 31 studies illustrating nervous system involvement by SARS-CoV-2 virus and 21 studies describing neurological involvement by other human coronaviruses. 31 SARS-CoV-2 articles (27 case reports and 4 case series) portrayed a wide spectrum of neurological manifestations in children involving both central nervous system (ADEM, encephalitis, seizures, stroke) as well the peripheral nervous system (GBS, transverse myelitis). Another group of children presenting with neurological manifestations with COVID-19 were those with MIS-C (multisystem inflammatory syndrome in children) which were described in 8 case reports. The authors found a wide variability in duration of follow-up and extent of evaluation amongst these studies. This study raises a rational concern that these children are at risk of long-term neurodevelopmental deficits which may not be apparent before discharge or during early follow-up. A comprehensive list of signs of potential neuro-developmental impairment across various age groups is also provided by the authors. As underscored by this article, there is a critical need for long-term neuro-developmental follow-up of these children by attaching them to developmental clinics under the collaborative care of pediatricians, pediatric neurologists and child psychologists.

A double-blind randomized, placebo-controlled clinical study of trofinetide in the treatment of fragile X syndrome (*Pediatric Neurol. 2020;110:30-41*)

Fragile X syndrome (FXS) is a neuro-developmental disorder characterised by >200 CGG repeats in the FMR1 gene. It has a significant prevalence of about 1 in 4000 males and 1 in 6000 females and there is no approved drug therapy for this disorder currently. This randomised controlled trial evaluated safety and tolerability of trofinetide in 72 adolescent and adult males with FXS. Trofinetide is an oral drug which is an analogue of amino-terminal tripeptide of insulin-like growth factor (IGF-1) which is postulated to improve symptoms of FXS by reducing neuro-inflammation, normalize dendritic morphology, reduce microglia activation and astrogliosis. Subjects were randomized to receive 35 mg/kg and 70 mg/kg trofinetide vs placebo BID for 28 days. Both doses were well tolerated and were found safe. Higher dose trofinetide was found to be efficacious in reducing key symptoms of FXS. As the duration of study was short and sample size was limited, larger trials are required to explore the efficacy of this potentially promising drug.

Clinical and imaging features of children with autoimmune encephalitis and MOG antibodies (*Neurol Neuroimmunol Neuroinflamm. 2020;7:e731*)

MOG abs (myelin oligodendrocyte glycoprotein antibodies) have been described typically in childhood central nervous system demyelinating disorders. Recently, these antibodies have been reported to be associated with autoimmune encephalitis (AE) with MRI features such as cortical and deep grey matter involvement in children and adults. This study describes a cohort of 10 children with AE and MOG abs. They presented at a median age of 8 years (range: 4-16 years) with encephalopathy (10/10) and a combination of headache, seizures and focal neurologic signs. Contrary to demyelinating disorders, none of the children had white matter involvement except juxta-cortical signal alterations in 6/10 children while all had cortical and deep grey matter involvement. Eight out of 10 children were treated with high dose intravenous methylprednisolone pulse therapy for 3-5 days and 1 child was given IV immunoglobulins. Nine out 10 children had favourable outcome (modified rankin scale 1) at 4 weeks except one child who had a residual focal deficit and had not received immunomodulation at the time of acute illness. This study highlights the crucial need of testing for MOG abs in all the children presenting with autoimmune encephalitis and prompt treatment with immunomodulation in such cases which is pivotal for a favourable outcome.

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RESEARCH PAPER

Targeted Audiological Surveillance Program in Campania, Italy

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Objective: To identify children with postnatal hearing loss, a structured monitoring system is needed. The goal of this study was to describe a targeted surveillance program in Italy to identify children with postnatal hearing loss. **Methods:** Between January, 2013, and December, 2016, all children who received bilateral 'pass' result at the newborn hearing screening, and who were identified as having at least one risk factor, were referred for targeted surveillance. The hospital records of these children were retrieved. **Results:** Among children enrolled, 66 were identified with permanent hearing loss. The most frequent risk factors were family history (35%), prematurity (25.5%), low birthweight (19.2%), severe hyperbilirubinemia (19%), prolonged ventilation (15%) and congenital infection (12.5%). **Conclusions:** An audiological surveillance program in newborns who 'pass' in neonatal screening, but have risk factors, is effective in identifying permanent postnatal hearing disorders.

Keywords: *Hearing screening, Newborn, Postnatal hearing loss, Risk factors.*

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Institution and improvement of universal newborn hearing screening (UNHS) has led to permanent hearing impairments being detected and treated as early as possible. The prevalence of permanent hearing impairment in newborn babies is approximately 0.5-1.5/1000, but it may increase up to 3.5-6/1000 children in school age [2]. The increase is due to the onset of postnatal hearing loss, which is missed in UNHS programs. The Joint committee on infant hearing screening (JCHI) recommends that an appropriate early identification and treatment of permanent hearing disorders, requires audiological surveillance in follow up on all newborns 'pass' but with risk factors for delayed/ progressive and acquired hearing loss [3].

We, herein, report the results of a targeted surveillance program based on selected risk factors in Campania region, Italy.

METHODS

A retrospective analysis of the audiological surveillance activities was performed for the period from January, 2013 to December, 2016. Well babies and neonatal intensive care unit (NICU) babies who received bilateral pass result during the newborn hearing screening and were neonatal intensive care unit one or more risk factor, were referred for targeted surveillance. The mean (SD) age of babies at the time of screening was 4 (2,1) weeks. The data of the present study were drawn from a database which included individual records for each child.

Since 2003, well-babies in Campania region are screened via two stage transient otoacoustic emission (TEOAE): The first in the course of the second or third day of life and the second between 3-4 weeks of age, if a refer result is obtained. TEOAE and automated auditory brainstem response (A-ABR) are reserved for infants under intensive care, prior to discharge. Infants who fail both screenings, either bilaterally or unilaterally, are referred to the nearest pediatric audiology service to perform a comprehensive audiology evaluation. In case of hearing impairment identification, a third level multidisciplinary diagnostic work-up together with appropriate management is provided by the regional reference center (RRC), at the audiology and vestibology unit of the neuroscience department of the university of Naples 'Federico II' [4]. Since 2013, this unit is also in charge of coordination of the audiological surveillance program according to the Position committee on infant hearing screening, 2007.

Children referred for surveillance appointment are accepted according to the following protocol: Audiological assessment in the third level center, every 6 months up to the age of 3 years and then annually up to

the age of 6 years in the presence of risk factors such as cytomegalovirus (CMV) and rubella intrauterine infections; every 6 months up to the age of 3 years, in children affected by syndromes associated with progressive or late-onset hearing loss e.g., Pendred syndrome, distal tubular renal acidosis, Waardenburg syndrome (type II), branchio-otorenal syndrome, Usher syndrome (type II and III), Stickler syndrome, CHARGE syndrome, Down syndrome, Turner syndrome, Alport syndrome, neurodegenerative disorders such as Hunter syndrome, sensory-motor neuropathies such as Friedreich ataxia, Charcot-Marie-Tooth syndrome); every 6 months up to the age of 2 years in case of family history of progressive permanent infant hearing loss, severe asphyxia; at 9-12 months in the case of prolonged ventilation for more than 5 days, craniofacial anomalies including cleft palate and, audiological evaluation at the immediate third level center in every phase of childhood or adolescence in case of chemotherapy, trauma, culture positive infections associated with sensorineural hearing loss, ototoxic drugs, reports from family pediatricians or other health workers and educators, or meningitis.

The audiological evaluation is done as per the age group of children. Test battery is click-auditory brainstem responses (ABR), transient evoked oto-acoustic emissions (TEOE), tympanometry at 3-9 months; TEOE, tympanometry, visual reinforcement audiometry at 9-12 months; and visual reinforcement audiometry, conditioned play audiometry and tympanometry at 3.5 years.

Degrees of hearing loss is based on the Bureau International for Audiophonology (BIAP) [5] classification viz normal (< 20 dB HL), mild (21-40 dB HL), moderate (41-70 dB HL), severe (71-90 dB HL) and profound (> 90 dB HL).

All families of children confirmed with hearing loss were offered a genetic evaluation and counselling. The evaluation, included a review of family history of specific genetic disorders or syndromes, genetic testing for gene mutations such as *GJB2*, *GJB6* (connexin-26 and 30), and syndromes commonly associated with early-onset hearing loss.

Statistical analyses: Descriptive statistics were used, and Pearson chi-squared analyses were performed in order to identify variables of significance.

RESULTS

The flow of all children referred to the targeted surveillance program is shown in **Fig. 1**. A total of 165416 children were eligible for UNHS (158386 'well babies' and 7030 'high risk'), of which 2752 (1.6%) children had a 'refer' result and underwent a comprehensive audiology evaluation. Another 2340 children had a 'pass' result, but with at least one risk factor and were referred for audiological surveillance program. Thus the recorded rate was 1.41% (2340/165416) in the period under investigation. With regards to individual risk factors, the largest proportion of referrals were generated from family history (35%), low birthweight (19.2%), prematurity (25.5%), severe hyperbilirubinemia (19%), prolonged ventilation (15%) and congenital infections (12.5%) (**Table I**).

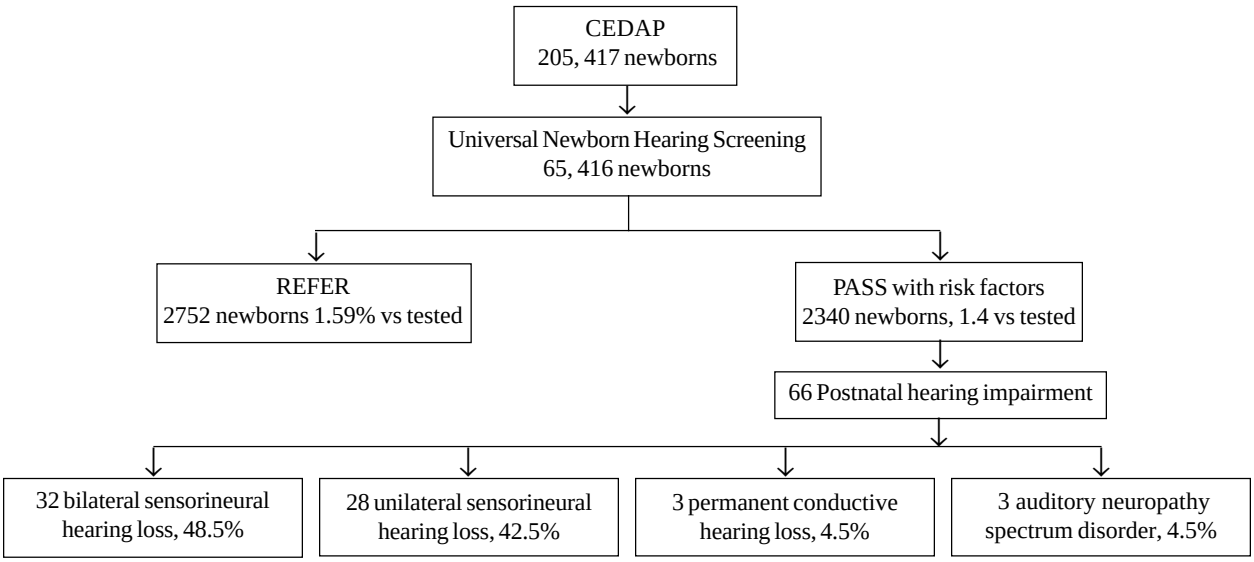

Fig. 1 *Results of the audiological surveillance program.*

WHAT THIS STUDY ADDS?

- We provide the results of a targeted surveillance program in Italy using a risk-factor list, providing information on prevalence of risk factors and characteristics of postnatal hearing loss.

Through the targeted surveillance program, a total of 66 (0.4%) children were identified with postnatal hearing loss - the most frequent risk factors were family history (26%), and prematurity (15%). Other less common risk factors reported were cleft palate (2 cases) and Pendred syndrome (1 case). Assisted prolonged ventilation (P=0.003), family history (P=0.003), cranio-facial anomalies (P=0.01), and congenital infections (P=0.02) were the significant risk factors for postnatal hearing loss.

Postnatal hearing loss exhibited the following types: 32 (48.5%) bilateral sensorineural hearing loss, 28 (42.5%) unilateral sensorineural hearing loss, and 3 (4.5%) each with permanent conductive hearing loss and auditory neuropathy spectrum disorder (ANSD). The degree of hearing loss was as follows: mild, 26 (39%); moderate, 10 (15%); severe, 6 (9%); and profound, 24 (37%). The mean (SD) age at diagnosis was 9.7 (7.8) months.

Table I Characteristics of Children in Targeted Follow-up Group and With Postnatal Hearing Loss

Characteristics	*Targeted follow-up group, n=2340*	*Postnatal hearing loss*
Male gender	1077 (46)	32 (48)
Number of risk factors		
1	1570 (67.1)	-
2	262 (11.2)	-
3	407 (17.4)	-
4	29 (1.2)	-
5	72 (3.1)	-
Type of risk factors		
Family history	819 (35)	17 (26)
Prematurity	596 (25.5)	10 (15)
Low birthweight	449 (19.2)	9 (13.6)
Hyperbirilubinemia	444 (19)	7 (10.6)
Prolonged ventilation	351 (15)	5 (7.6)
Congenital infections	292 (12.5)	5 (7.6)
Neonatal asphyxia	187 (8)	4(6)
Craniofacial anomalies	140 (6)	2 (2.5)
Syndromes	112 (4.8)	1 (1.5)
Pediatricians/ caregivers reporting	70 (3)	0
Bacterial meningitis	5 (0.2)	0 (0)

DISCUSSION

Out region's surveillance program aims to detect postnatal and progressive hearing loss to avoid after-effects due to late diagnosis [6,7]. In the present work, we have used the list of risk factors proposed by JCIH in 2007 replacing the item 'entry in NICU' with prematurity (<37 weeks) and low birthweight (<2500g), in order to avoid too many referrals in the program for these risk factor and to make the follow-up protocol more feasible, effective and selective, and extracorporeal membrane oxygenation (ECMO) was replaced with severe asphyxia [8,9]. We also redefined the audiological protocol proposed in relation to both the timing for each risk factor and the methodology by age group, as described above.

Our data confirm the increase of permanent hearing disorders in the postnatal period; which, in our evaluation, reaches a rate of 2.6%. In the analyzed sample, a prevalence of permanent hearing impairment of a mild degree is evident. The immediate identification is particularly relevant because of the negative impact on the linguistic and curricular outcomes of this hearing loss [10]. In contrast to Beswick, et al. [11], where neonatal asphyxia was the primary cause of postnatal hearing loss, we found family history, congenital infections, and prolonged mechanical ventilation as significant risk factors.

The epidemiological and clinical aspects of the hearing impairment identified in the postnatal period in the current study supports the need and effectiveness of audiological surveillance during early childhood [6,7,12]. Audiological surveillance allowed us to identify not only cases of progressive hearing loss that probably arose in the postnatal period, but also congenital forms that had avoided the neonatal auditory screening, as the high presence of mild forms exhibits. The significant risk factors identified in this study need further evaluation in other regions and different populations.

Disclaimer: The views in this article are those of the authors and do not necessarily represent the official views of the Disability Research and Dissemination Center or the Centers for Disease Control and Prevention.

Ethical clearance: University of Naples 'Federico II' Ethics Committee; No. 56/18, dated March 26, 2018.

Contributors: RM: conceptualized and designed the study and drafted components of the initial and final manuscript and had a major role in the written manuscript as submitted; AF, EM:

participated in the conceptualization and design of the study, oversaw the collection of the data; EB: conducted the statistical analyses; CM, CL, GA: participated in the review of the literature, assisted in data collection, drafted sections of the initial manuscript, and participated in editing of the final manuscript as submitted; ME: conducted the initial literature review; FT: supplied critical background material for the study, and critically reviewed the manuscript. All authors approved the final manuscript as submitted.
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CLIPPINGS

Pharmacological and neurosurgical interventions for individuals with cerebral palsy and dystonia: A systematic review update and meta-analysis (*Dev Med Child Neurol. 2021;dmcn.14874*)

This systematic review and meta-analysis is an update to the previous systematic review up to December 2015, with 19 new studies identified from January 2016 to May 2020. This review included a total of 46 studies comprising 915 participants. Awareness about the evidence on anti-dystonia measures is crucial for all pediatric medicine practitioners as it is a very common co-morbidity in children with cerebral palsy and other chronic neurological disorders.

Very low certainty evidence was found for clonidine, BoNT (botulinum toxin), ITB (intra-thecal baclofen) and DBS (deep brain stimulation) as anti-dystonia measures. Little to no effect on dystonia was found in randomized as well as non-randomized studies for trihexiphenidyl, one of the most commonly prescribed drug for dystonia. No studies evaluating benzodiazepines, gabapentin and medical cannabis were found during the review period. Evidence for levodopa was limited to a single randomized crossover trial. A new publication has suggested improvement in dystonia with clonidine. This study also highlights that both pharmacological and surgical measure should be exercised with caution as trihexiphenidyl, clonidine, BoNT, DBS and ITB may increase the adverse events.

Safety and efficacy of tocilizumab versus azathioprine in highly relapsing neuromyelitis optica spectrum disorder (TANGO): an open-label, multicentre, randomised, phase 2 trial (*Lancet Neurol. 2020;19:391-401*)

This multicentric, phase 2 trial explored the efficacy of intravenous tocilizumab (8 mg/kg every 4 weeks) as compared to oral azathioprine (2-3 mg/kg/day), which is the standard therapy for long term immunosuppression for patients with neuromyelitis optica spectrum disorder (NMOSD). This study enrolled 118 adults (59 in each arm) who were evaluated for time to first relapse as the primary outcome measure. Median time to first relapse was longer for tocilizumab vs azathioprine (78.9 weeks vs 56.7 weeks, P=0.0026) in full analysis set. Eighty nine percent of patients in tocilizumab group remained relapse free at the end of the study as compared to 56% in the azathioprine group in the per-protocol analysis. This study highlighted tocilizumab as a safe and efficacious choice for long term immunosuppression for patient with NMOSD. These findings need to be validated in pediatric age group with an adequate sample size.

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RESEARCH PAPER

Next-Generation Sequencing for Congenital Nephrotic Syndrome: A Multi-Center Cross-Sectional Study from India

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Objective: Information on etiology of congenital nephrotic syndrome in non-Caucasian populations is limited. This study aimed to determine the genetic basis of congenital nephrotic syndrome in Indian patients. **Methods:** In this observational, cross-sectional study, whole exome sequencing was performed on samples from all children diagnosed with congenital nephrotic syndrome, presenting at centers collaborating in a nationwide registry and biorepository. Analysis was targeted to focus on reported or novel, pathogenic or likely pathogenic variants in 89 genes implicated in etiology of nephrotic syndrome. Sanger sequencing was used to confirm disease-causing variants in patients and allelic segregation of compound heterozygous variants in samples from parents. Inheritance of a shared haplotype was analyzed among ten individuals carrying the most common variant. **Results:** During 2017-2019, 34 patients with congenital nephrotic syndrome were screened. Consanguinity and similar illness in siblings were reported in eleven patients each. Homozygous or compound heterozygous, pathogenic or likely pathogenic variants were found in *NPHS1* in 24 cases, including one novel variant. One patient each had homozygous pathogenic or likely pathogenic known or novel variant in *NPHS2*, *PLCE1, OSGEP* and *LAMB2* genes. Patients with *OSGEP* and *LAMB2* mutations had phenotype typical of Galloway Mowat and Pierson syndromes, respectively. Three variants in *NPHS1* were common to 16 individuals. One reported variant in exon 19 (c.2600G>A; p.Gly867Asp) appears to share a common founder. **Conclusion**: A genetic cause was determined for 82.4% patients with congenital nephrotic syndrome. Variants in *NPHS1* are most common in Indian patients and founder mutations might be present.

Keywords: *Nephrin, podocin, Galloway Mowat syndrome, Pierson syndrome, NPHS1*

Congenital nephrotic syndrome (NS) is a rare condition, characterized by nephrotic range proteinuria, hypoalbuminemia and edema before 3 months of age. Most patients show morbidities related to edema, infections and/or thrombosis, and progression to end stage renal disease (ESRD) in early childhood [1]. An inherited basis is reported in 60-80% patients; variants in *NPHS1*, which are most frequent and also cause the Finnish type of congenital NS [2], along with variants in *NPHS2*, *PLCE1*, *LAMB2* and *WT1*, result in defects affecting proteins in the podocyte slit diaphragm, actin cytoskeleton or transcription regulation [3-5]. Existing reports on variants in Asian patients are single-center and retrospective, screening for few genes [6-10]. We describe here the results of next-generation sequencing (NGS) in infants with congenital NS, enrolled prospectively from April, 2017 to June, 2019, in a multicenter collaboration on nephrotic syndrome.

METHODS

Following ethics approval and informed parental consent, clinical details and blood samples were collected from patients with congenital NS, diagnosed at seven tertiary care centres in the country. Diagnosis required the confirmation of nephrotic range proteinuria (spot urine protein to creatinine ratio >2.0 mg/mg or dipstick 3+/4+ on three occasions), hypoalbuminemia (serum albumin <3.0 g/dl) and edema beginning below 3-months of age. Intrauterine infections and structural renal anomalies were excluded by appropriate serology and ultrasonography, respectively. In consonance with current practice worldwide, kidney biopsy was not performed and echocardiography was performed if cardiac examination was abnormal. Management involved the use of furosemide (1-2 mg/kg daily, as

indicated), enalapril (0.3-0.4 mg/kg/day orally), intravenous infusions of albumin (1-2 g/kg once every 7-14 days), and supplements of thyroxine (5-10 µg/kg/day) and vitamins, while ensuring adequate nutrition. Parents were counselled regarding outcomes including risk of progression to end stage kidney disease, and families opted for a palliative care plan due to costs of kidney replacement therapy.

The methodology of NGS, performed at Institute of Genomic and Integrative Biology, Delhi, is detailed in **Web Methods**. Whole exome sequencing (WES) was performed using the Illumina HiSeq2000 or NovaSeq platforms, sequenced reads were mapped and aligned to the reference genome (GRCh37; hg19), and called and annotated variants in 89 genes associated with nephrotic syndrome (**Web Table SI**) [3,11-14] were prioritized based on rarity (minor allele frequency, MAF <0.1%), novelty in population databases [15-17], prediction of deleteriousness by *in silico* tools, and if previously reported with disease [18]. Only pathogenic and likely pathogenic variants, according to criteria of the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines [18,19] were considered causative, and were validated by Sanger sequencing. Sanger sequencing on parents' samples was used to confirm allele segregation for compound heterozygous variants. Haplotype studies were performed to determine if the *NPHS1* variant c.2600G>A (p.Gly867Asp) that segregated in 10 of 34 patients occurred on a common genetic background, suggesting inheritance from a common ancestor (founder mutation) (**Web Methods**) [20].

Statistical analyses: Data was summarized as median (interquartile range, IQR) for continuous variables and percentage with 95% confidence interval (CI) for dichotomous variables. Assuming 70% prevalence of pathogenic or likely pathogenic variations in genes encoding key podocyte proteins in patients with congenital nephrotic syndrome [1,3,12,13], 21 patients were required to be enrolled for a precision of 20%, at power of 80% and alpha error of 5%.

RESULTS

Samples were collected from 34 unrelated patients (53% boys) with congenital NS diagnosed at 7 centers across India. Onset of edema was at median age of 20 (IQR 15-45) days of life, and was associated with anasarca (91.2%), oliguria (41.2%), poor feeding (35.3%), seizures (32.3%), hypovolemia (23.5%), severe infections (20.6%) and/or lethargy (8.8%). Ten (29.4%) patients were born premature and 13 (38.2%) had low birth weight (**Web Table SII**). Consanguinity and similar illness in siblings were reported in 11 (32.4%) cases, each.

Median weight for age standard deviation score (SDS) was -3.1 (IQR -4.1, -1.9), length for age SDS was -3.9 (IQR -4.6, -2.2) and head circumference SDS was -3.2 (IQR -4.5, -2.2). Seven (20.6%) patients had hypertension. Isolated extrarenal features were observed in 9 patients (**Web Table SII**), while one patient each had features of Galloway-Mowat and Pierson syndrome. One patient had albinism and microcephaly and history of sibling death with similar symptoms.

The median blood level of albumin was 1.2 (IQR 0.9-1.4) g/dL, cholesterol 274 (234-349) mg/dL, creatinine 0.4 (0.3-0.7) mg/dL, and estimated glomerular filtration rate (eGFR) 60 (28.3-96) mL/minute per 1.73 m^2 [21]. Seven (20.6%) patients had eGFR <30 mL/minute per 1.73 m^2 at evaluation. Three (8.8%) patients had enlarged kidneys without hydronephrosis or venous thrombosis. There was no history of significant teratogenic drug intake during pregnancy or evidence of intrauterine infection.

WES with mean coverage of ≥30x (**Web Table SIII)** returned 16804 variants, of which 1370 variants were present in one or more of the targeted genes (**Fig. 1**). After filtering, 91 variants were shortlisted (***Web Table* SIV**), of which 22 variants were prioritized in 28 patients (**Table I*;* Web Fig. S1)**. Pathogenic and likely pathogenic variants were inherited as homozygous and compound heterozygous variations in 20 and 8 patients, respectively. A monogenic cause was thus established in 82.4% (95% CI 66.9% to 92.5%) of 34 patients with congenital NS. Most variants were conserved across species (**Web Fig. S2**).

Variants in *NPHS1* were most common, including 16 reported [11,12,14,15,22-35] and two novel variants, segregated in 24 patients as homozygous (*n*=16) and compound heterozygous (*n*=8) variants (**Table I**). Reported variations included 7 pathogenic and 9 likely pathogenic variants. One novel homozygous variant in ID#181 was classified as likely pathogenic, while another novel *NPHS1* variant that segregated as compound heterozygous in ID#8, was assigned as pathogenic. **Fig. 2** indicates the distribution of defects in *NPHS1* across the structure of nephrin.

One previously reported [11,14] likely pathogenic *NPHS1* variant in exon 19 (c.G2600A; p.Gly867Asp) was inherited as homozygous in 7 and heterozygous in 3 patients from different ethnic and regional backgrounds, without any specific phenotype (**Tables I** and **SII**). Two other reported variations, p.Arg1160Ter [11,14,27] and p.Arg367Cys [14,25,27], were common to three patients each (**Table I**). In patients with *NPHS1* variants, atrial septal defect was seen in two patients, and developmental delay, facial dysmorphism, clubbing, café

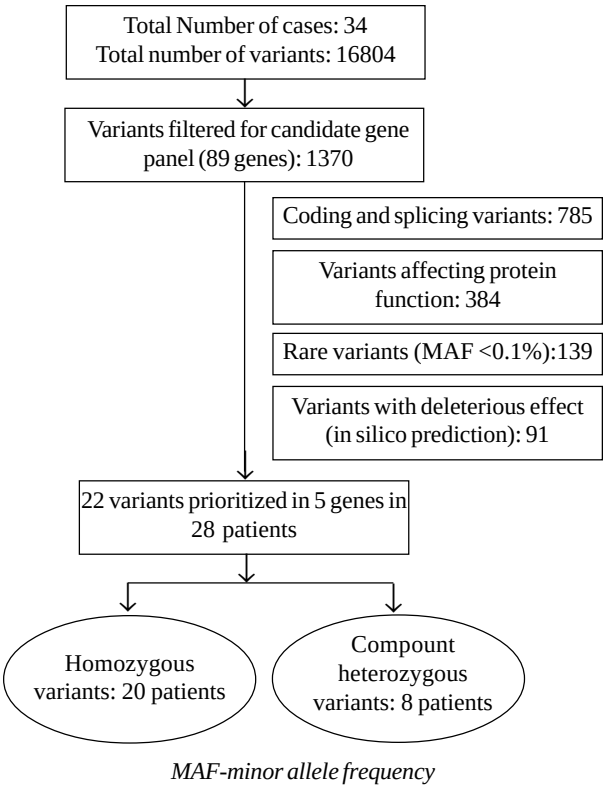

Fig. 1 Flowchart for variant filtering after whole exome sequencing.

au lait spots, hirsutism and aqueductal stenosis in one patient each (**Web Table SII**).

One patient each had homozygous likely pathogenic variants in *NPHS2* [34] and *OSGEP* [36], associated with an atrial septal defect and Galloway-Mowat syndrome, respectively. One patient each had novel pathogenic homozygous variations in *PLCE1* and *LAMB2* genes; the latter was associated with phenotype consistent with Pierson syndrome.

No variants were prioritized in two patients; four patients had heterozygous variations that were of unknown significance (**Web Table SIV**). Patients with causative variations also had additional heterozygous variations (**Web Table SIV**).

There were no differences in sex ratio, age at onset of symptoms, levels of serum albumin or estimated GFR between patients with *NPHS1* variations and those with other or no significant variations (P>0.05 each).

Forty-four of 900 single nucleotide polymorphisms (SNPs) (**Web Table SV**) in the region (±500 kbp) flanking the c.2600G>A (Gly867Asp) were selected for haplotype analysis in 33 patients. All 17 alleles carrying the c.2600G>A variant (homozygous in 7 and heterozygous in 3 patients) shared a core haplotype in the 500 kbp region between rs2230181 to rs466452 (**Web Table SVI).** Thirteen of 17 alleles also shared a core haplotype extending to 800 kbp length. The 500 kbp core haplotype was observed in only one of 49 non-mutant chromosomes, suggesting a founder effect.

DISCUSSION

There is significant heterogeneity in prevalence of inherited defects across studies (**Web Table SVII**) [6-10,12,22-26,34]. Variants in *NPHS1* predominate even in non-Finnish cohorts, and contributions by *NPHS2*, *WT1* and *LAMB2* defects differ widely across populations. In the present study, the use of NGS enabled a diagnosis in 82% of 34 patients. These findings are unlike previous studies from non-Caucasian populations that report lower rates of inherited defects, perhaps due to focused testing including a few genes **(Web Table SVII**).

Two founder deletion mutations in *NPHS1*, accounting for the majority of cases of Finnish type of congenital nephrotic syndrome, were not observed in our patients, similar to reports from non-Finnish populations [2,3,6-10]. Over 200 *NPHS1* mutations are described worldwide in non-Finnish populations [29,32]. In our report, homozygous and compound heterozygous mutations in *NPHS1* accounted for 70.6% of cases of congenital NS, and 85.7% of cases with an identified genetic etiology. This proportion is higher than previous reports from Asia, in which *NPHS1* mutations accounted for 22-67% of cases, but similar to proportions reported in series including non-Finnish populations (**Web Table SVII**).

As shown in **Fig. 2**, variants in *NPHS1* were distributed all over the protein. Three patients shared the variant p.Arg1160Ter, responsible for premature truncation of protein in the intracellular domain that interacts with podocin. This variant, a founder mutation in Maltese patients, is associated with a different allele in Asian patients [25]. While Koziell, et al. reported a mild phenotype in affected girl infants [25], we and other authors [24,35] found a severe phenotype, irrespective of gender, indistinguishable from other *NPHS1* mutations. Three patients carried a variant (c.1099C>T; p.Arg367Cys), reported previously as a founder mutation from India [12]. One *NPHS1* variant, c.2600G>A (p.Gly867Asp), that translates into a change in the immunoglobulin-like domain 8, found in 10 unrelated patients from five states in north India (**Web Table SII**, **Table I** and ***Fig.* 1**), has been reported from India, Pakistan and Saudi Arabia [8,11,14,37], but not from east Asia [6,7,9] or Europe. Using statistical tools considered more efficient that conventional haplotyping [20], we show

Table I Pathogenic and Likely Pathogenic Variants in Patients With Congenital Nephrotic Syndrome[a]

Cene (chromosome); Chromosomal coordinate; change	*Exon*	*Variant change*		*ACMG# category*[b]	*Patient ID*[c]	*Reference*
		cDNA change	*Protein change*			
NPHS1 (19)						
36341349 T>C	5	c.527-2A>G		Pathogenic[b5]	165	33
36341342 G>A	5	c.532C>T	p.Gln178Ter	Pathogenic	8	26, 29, 34
36340176 G>A	7	c.802C>T	p.Arg268Ter	Pathogenic	80	26, 30
36337056 G>T	12	c.1481C>A	p.Ser494Ter	Pathogenic	180	29,31
36335078_36335079delGT	16	c.2138_2139delAC	p.Asp713Glyfs*12	Pathogenic[b6]	8	Novel
36330221 G>C	22	c.3027C>G	p.Tyr1009Ter	Pathogenic	**168**	23, 34
36321958 G>A	27	c.3478C>T	p.Arg1160Ter	Pathogenic	**150, 169,** 173	11,14,22,23,24,25,26,27,29,32, 35
36317522 TC>T	29	c.3619delG	p.Glu1207Lysfs*30	Pathogenic[b6]	4	ClinVar
36341889 G>A	4	c.500C>T	p.Pro167Leu	Likely pathogenic	267	11, 22
36340541-36340548 delCCGGGGTGinsAA	6	c.614_621delinsTT	p.Thr205_Arg207delinsIle		180,228 Likely pathogenic[b4]	22, 26,35
36339610 G>A	9	c.1099C>T	p.Arg367Cys	Likely pathogenic	**59**, 165, 267	14,22,25,26,29,30,33, 35
36339251 G>A	10	c.1219C>T	p.Arg407Trp	Likely pathogenic[b2]	**85**	22
36336350 T>C	14	c.1850A>G	p.His617Arg	Likely pathogenic[b1]	80	26,27,29,30
36335272 G>A	15	c.2020C>T	p.Pro674Ser	Likely pathogenic[b1]	**163**	24
36333370 G>T	18	c.2417C>A	p.Ala806Asp	Likely pathogenic[b2]	**157**	14, 26, 27,29
36333089 C>T	19	c.2600G>A	p.Gly867Asp	Likely pathogenic[b1]	4, **40, 47, 146, 162,** 173**, 196, 201**, 228, **235**	11,14, 34
36332715 A>G	20	c.2717T>C	p.Ile906Thr	Likely pathogenic[b1]	**181**	Novel
36322049 C>T	27	c.3388-1G>A		Likely pathogenic	**18**	26, 27
NPHS2 (1)						
179530456 C>T	3	c.419G>A	p.Gly140Glu	Likely pathogenic[b1]	**28**	34
PLCE1 (10)						
96058156 GC>AT	23	c.4264C>T	p.Gln1422Ter	Pathogenic	**46**	Novel
OSGEP (14)						
20920566 T>A	2	c.157A>T	p.Ile53Phe	Likely pathogenic[b7]	**154**	36
LAMB2 (3)						
49168499 G>A	8	c.799C>T	p.Arg267Ter	Pathogenic	**X1**	Novel

[a]Six patients did not have any pathogenic or likely pathogenic variants. ACMG-American College of Medical Genetics. [c]Patient IDs indicated in bold had homozygous variation; those not in bold were heterozygous variants. [b]Criteria were manually adjusted:[1]PM1, PM2, PP3& PP4; [2]PM1, PM2, PP4 & PP5; [3]PM1, PM2 & PP4; [4]PM1, PM2, PM3, PP4 & BP4; [5]PVS1, PM2, PP3, PP4; [6]PVS1, PM2, PP4; [7]PM1, PM2, PP2, PP4 & BP4

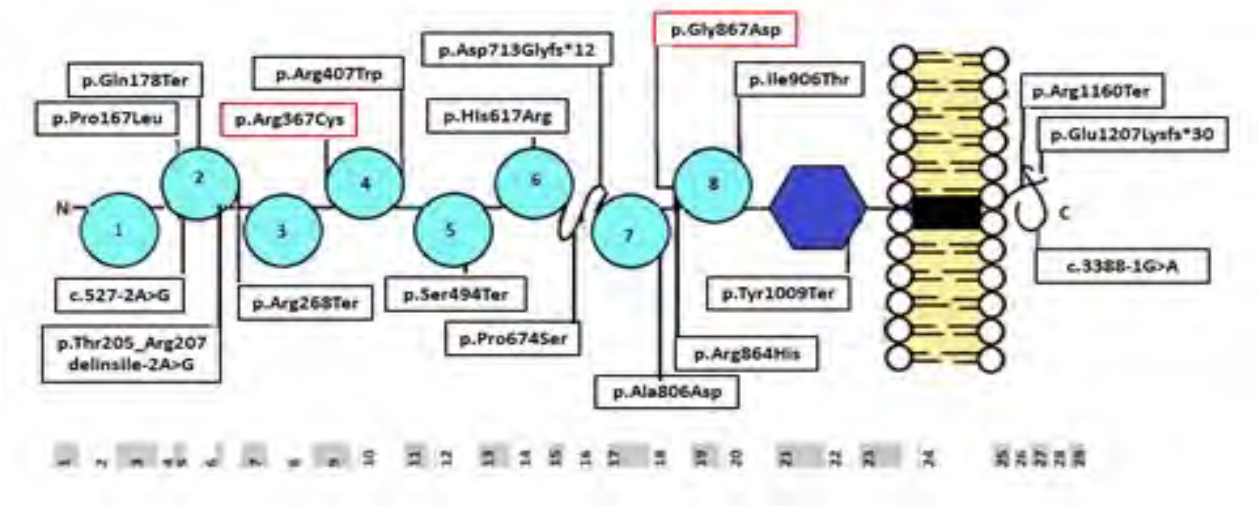

Fig. 2 Localization of novel variations and known mutations in the translated nephrin protein, comprised of eight extracellular immunoglobulin (Ig) -like domains (semi-circles), a fibronectin type III-like module (hexagon), a transmembrane domain (black rectangle) and a C-terminal (C) cytoplasmic domain (curled line). The bottom panel indicates the exons coding for the corresponding protein domains. Note that the 18 variations observed were spread throughout the protein. The variations with dotted lines are known or speculated to be founder mutations.

that c.2600G>A is possibly a founder mutation, as suggested by the lack of genetic variation in the 500-800 kbp length flanking regions [38]. The differences in frequency of the shared haplotype in various ethnicities in the 1000 genome database suggests a European origin for the mutation **(Supp. Table SVIII)** [15]. Our hypothesis requires confirmation by examining for the same shared haplotype in previously reported patients with the p.Gly867Asp mutation.

Mutations in *NPHS2* and *WT1* account for 0-51% and 0-40% cases, respectively, across populations, though *NPHS2* variants are uncommon in Asia (**Supp. Table SVII**). In this cross-sectional study, only one patient had homozygous mutations in *NPHS2*, and none had variants in *WT1*. Given the small study size, these findings have limited generalisability.

Confirming previous findings, we failed to find specific phenotypic associations in patients with *NPHS1*, *NPHS2* and *PLCE1* mutations [4,26,39]. The lone patient with homozygous *LAMB2* variant had findings of Pierson syndrome while another had Galloway-Mowat syndrome secondary to *OSGEP* mutation [36]. The latter patient had the same mutation and phenotype as an infant of Pakistani ethnicity described previously [36].

The present series underscores the utility of providing a genetic etiology in patients with congenital NS, thereby facilitating prenatal counseling and testing in subsequent pregnancies. One *NPHS1* mutation is hypothesized to have a founder effect in Indian population. Information on long term outcomes, including post-transplantation, is lacking since most children were lost to follow up after families chose a palliative care plan. Despite being a multicenter study, the findings of the relatively small sample size might not be generalizable.

Note: Supplementary material related to this study is available with the online version at *www.indianpediatrics.net*

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WHAT IS ALREADY KNOWN?

- Genetic defects account for 60-80% of cases with congenital nephrotic syndrome
- Mutations in *NPHS1* are most common in Caucasians; *WT1* and *LAMB2* variants are probably more common in Asian patients

WHAT THIS STUDY ADDS?

- Genetic defects are present in more than 80% patients with congenital nephrotic syndrome in India
- Mutations in *NPHS1* account for more than 80% of patients with an inherited basis
- Common variants in *NPHS1* are those that are known (c.1099C>T; p.Arg367Cys) or speculated (c.2600G>A; p.Gly867Asp) to be founder mutations.

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SUPPLEMENTARY METHODS

DNA extraction: DNA was extracted from EDTA blood using QIAamp® DNA Blood Mini Kit (Qiagen, Germantown, MD), as per manufacturer instructions. The quality and the quantity of DNA extracted was assessed using NanoDrop™ spectrophotometer (Thermo Scientific, Wilmington, DE) and 1% gel electrophoresis before next generation sequencing.

Whole exome sequencing (WES): DNA libraries were prepared using 100 ng of DNA, physically sheared on an ultrasonicator (Covaris), followed by ligation of adapter sequences on to fragmented DNA to generate indexed libraries, and exome enrichment using TruSeq Exome kit (Illumina, San Diego, CA), as per manufacturer protocol. Enriched libraries were quantified by Qubit fluorometer (ThermoFisher) and their size distribution measured using Bioanalyzer (Agilent). Three of 34 samples underwent cluster generation using Illumina Cbot followed by paired end sequencing (2x100 bp) using flowcell v3 on Illumina HiSeq2000 platform. The remainder were sequenced (2x150 bp) on Illumina NovaSeq platform using S2/S4 flowcell.

Processing of sequenced reads: Paired-end sequenced reads were processed using the Dynamic Read Analysis for GENomics Bio-IT (DRAGEN, Illumina) platform. The reads were demultiplexed and then mapped and aligned to the reference genome (GRCh37; hg19) using the seed generation algorithm followed by Smith-Waterman algorithm. This was followed by variant calling using Haplotype Caller (Dragen), merging of individual variant call files (VCF) using VCFtools (*vcftools.sourceforge.net*) and annotation of merged VCFs using ANNOVAR (*annovar.openbioinformatics.org*).

Variant prioritization: Based on literature search, 89 genes were considered relevant for genetic testing in nephrotic syndrome [i,ii,iii,iv,v]. The list of genes, along with the mean coverage of exonic regions, is provided in **Suppl. Table S1**. Variants in these genes were considered potentially disease causing if they fulfilled one of the following criteria: *(i) rare and deleterious,* with rarity defined as minor allele frequency (MAF) of less than 0.1% in the population databases of 1000 Genomes Project [vi], Exome Aggregator Consortium (Exac) [vii] and Genome Aggregation Database (gnomAD) [viii]; and deleteriousness predicted by assertion of pathogenicity on at least two computational tools, including Polymorphism Phenotyping v2 (PolyPhen2; *http://genetics.bwh.harvard.edu/pph2/*), Sorting Intolerant from Tolerant (SIFT; *https://sift.bii.a-star.edu.sg/*), Mutation taster v2 (*http://www.mutationtaster.org/ChrPos.html*), Combined Annotation Dependent Depletion (CADD; *https://cadd.gs.washington.edu/*) and Genomic Evolutionary Rate Profiling score

(GERP_RS;*http://varianttools.sourceforge.net/Annotation/dbNSFP*), Eigen (*https://omictools. com/eigen-tool*), and where relevant, Human Splicing Finder v3.1 (*http://umd.be/HSF3/*); *(ii) novel and deleterious*, with novelty defined by absence in the two population databases as well as in the Database of Single Nucleotide Polymorphism (DbsNP; *https://www.ncbi.nlm.nih.gov/snp/*) and ClinVar (*https://www.ncbi.nlm.nih.gov/clinvar/*); or *(iii) reported,* in causative association with disease (congenital or steroid resistant nephrotic syndrome) in ClinVar (*https://www.ncbi.nlm.nih.gov/clinvar*) or Human Genome Mutation Database (HGMD; *http://www.hgmd.cf.ac.uk*), particularly if reported as 'pathogenic' or 'likely pathogenic' in ClinVar.

Variants shortlisted based on above criteria were excluded if any of the following conditions were fulfilled: *(i) high (≥0.1%) MAF in south Asian population* of ExAC; *(ii) failing to have causative phenotype*, such as a variant called in heterozygous state in a gene following autosomal recessive pattern of inheritance [ix]; or *(iii) low depth:* variant with read depth of <10x. Prioritised variants were classified from benign to pathogenic using the web-based clinical INTERpretation of VARiants (wINTERVAR; *http://wintervar.wglab.org/*), with or without modifications to follow the criteria outlined by the 2015 guidelines of the American College of Medical Genetics and Genomics (ACMG) [x].

Variant validation: Variants considered causative of disease were validated by Sanger sequencing using the ABI3730 genetic analyzer (Applied Biosystems). Sanger sequencing on parents' samples was used to confirm allele segregation for compound heterozygous variations.

Haplotype analysis of p.Gly867Asp mutant allele: The NGS data was used to obtain all single nucleotide polymorphisms flanking the mutation (±500 kbp). Variants with MAF ≥0.05% and significant difference in frequency (P<0.05) in patients with and without the mutation (p.Gly867Asp) were selected for haplotype analysis by Phase v2.1 (*http://stephenslab.uchicago.edu/phase/download.html*) to obtain haplotypes segregating with Gly867 and Asp867 associated alleles.

Supplementary Table SI Panel of 89 genes examined for association with congenital nephrotic syndrome along with coverage

Gene	*Disease*	*Inheritance*	*Transcript*	*Mean Coverage*
ACTN4	Glomerulosclerosis, focal segmental, 1	AD	NM_004924.5	109.40
ALG1	Congenital disorder of glycosylation, type Ik	AR	NM_019109.4	126.06
ALMS1	Alstrom syndrome	AR	NM_015120.4	104.55
ANKS6	Nephronophthisis 16	AR	NM_173551.4	46.96
ANLN	Focal segmental glomerulosclerosis 8	AD	NM_001284301.2	61.67
APOL1	End-stage renal disease, nondiabetic, susceptibility to Glomerulosclerosis, focal segmental, 4, susceptibility to	-	NM_145343.2	51.61
ARHGAP24	-	-	NM_001025616.2	111.73
ARHGDIA	Nephrotic syndrome, type 8	AR	NM_001301242.1	44.86
AVIL	-	-	NM_006576.3	65.40
CD151	Nephropathy with pretibial epidermolysis bullosa and deafness	-	NM_001039490.1	49.87
CD2AP	Glomerulosclerosis, focal segmental, 3	-	NM_012120.2	124.88
CFH	Complement factor H deficiency (hemolytic uremic syndrome, atypical, susceptibility to, 1)	AR|AD	NM_000186.3	134.46
CLCN5	Nephrolithiasis, type I: Proteinuria, low molecular weight, with hypercalciuric nephrocalcinosis	XLR|XLR	NM_001127898.3	77.52
COL4A1	Angiopathy, hereditary, with nephropathy, aneurysms, and muscle cramps	AD	NM_001845.5	126.02
COL4A3	Alport syndrome 2, autosomal recessive; Alport syndrome 3, autosomal dominant; hematuria, benign familial	AR|AD|AD	NM_000091.4	131.02
COL4A4	Alport syndrome 2, autosomal recessive| hematuria, familial benign	AR|AD	NM_000092.4	118.25
COL4A5	Alport syndrome 1, X-linked	XLD	NM_000495.4	90.51
COQ2	Coenzyme Q10 deficiency, primary, 1	AR	NM_015697.7	81.81
COQ6	Coenzyme Q10 deficiency, primary, 6	AR	NM_182476.2	149.02
COQ7	Coenzyme Q10 deficiency, primary, 8	AR	NM_016138.4	53.76
COQ8B	Nephrotic syndrome, type 9	AR	NM_001142555.2	52.54
COQ9	Coenzyme Q10 deficiency, primary, 5	AR	NM_020312.3	130.77
CRB2	Focal segmental glomerulosclerosis 9; ventriculomegaly with cystic kidney disease	AR|AR	NM_173689.6	41.61
CUBN	Finnish type	AR	NM_001081.3	136.24
CYP11B2	Hypoaldosteronism, congenital, due to CMO I deficiency; hypoaldosteronism, congenital, due to CMO II deficiency	AR|AR	NM_000498.3	121.62
DGKE	{Hemolytic uremic syndrome, atypical, susceptibility to, 7}| Nephrotic syndrome, type 7	AR|AR	NM_003647.2	58.76

E2F3	-	-	NM_001949.4	61.62
EMP2	Nephrotic syndrome, type 10	AR	NM_001424.5	45.32
EXT1	Chondrosarcoma| Exostoses, multiple, type 1	AR|AD	NM_000127.2	114.96
FAT1	-	-	NM_005245.3	75.17
FN1	Glomerulopathy with fibronectin deposits 2	AD	NM_001306129.1	127.17
G6PC	Glycogen storage disease	AR	NM_000151.3	126.16
GATA3	Hypoparathyroidism, sensorineural deafness, and renal dysplasia	AD	NM_001002295.1	94.05
GFND1	Glomerulopathy with fibronectin deposits 1	AD	MIM:137950	-
GLA	Fabry disease| Fabry disease, cardiac variant	X-linked	NM_000169.2	92.44
IGAN1	{IgA nephropathy, susceptibility to, 1}	?AD	MIM:161950	-
IGAN2	{IgA nephropathy, susceptibility to, 2}	?AD	MIM:613944	-
INF2	Glomerulosclerosis, focal segmental, 5	AD	NM_001031714.3	63.42
ITGA3	Interstitial lung disease, nephrotic syndrome, and epidermolysis bullosa, congenital	AR	NM_002204.3	52.16
ITGB4	Epidermolysis bullosa of hands and feet| Epidermolysis bullosa, junctional, non-Herlitz type| Epidermolysis bullosa, junctional, with pyloric atresia	AD|AR|AR	NM_001256876.1	101.91
KANK1	Cerebral palsy, spastic quadriplegic, 2	-	NM_001136191.2	70.34
KANK2	Nephrotic syndrome, type 16	AR	NM_001320269.1	45.10
KANK4	-	-	NM_006014.4	72.41
LAGE3	Galloway-Mowat syndrome 2, X-linked	XLR	NM_002292.3	24.05
LAMB2	-	-	NM_170708.3	126.50
LMNA	Cardiomyopathy, dilated, 1A| Charcot-Marie-Tooth disease, type 2B1| Emery-Dreifuss muscular dystrophy 2, autosomal dominant| Emery-Dreifuss muscular dystrophy 3, autosomal recessive| Heart-hand syndrome, Slovenian type| Hutchinson-Gilford progeria| Lipodystrophy, familial partial, type 2| Malouf syndrome| Mandibuloacral dysplasia| Muscular dystrophy, congenital| Restrictive dermopathy, lethal	AD|AR|AD|AR|AD| AR,AD|AD| AD|AR|AD|AR	NM_001282626.1	108.23
LMX1B	Nail-patella syndrome	AD	NM_001174146.1	56.96
LRP2	Donnai-Barrow syndrome	AR	NM_004525.2	130.34
MAFB	Multicentric carpotarsal osteolysis syndrome	AD	NM_005461.4	49.48
MAGI2	Nephrotic syndrome, type 15	AR	NM_001301128.1	66.23
MED28	-	-	NM_025205.4	79.14
MEFV	Familial Mediterranean fever | Familial Mediterranean fever	AD|AR	NM_000243.2	93.60
MT-TL1	-	-		-
MUC1	Medullary cystic kidney disease 1	AD	NM_002456.5	62.84

MYH9	Deafness, autosomal dominant 17| Macrothrombocytopenia and granulocyte inclusions with or without nephritis or sensorineural hearing loss	AD|AD	NM_002473.5	112.51
MYO1E	Glomerulosclerosis, focal segmental, 6	AR	NM_004998.3	125.65
NEIL1	-	-	NM_001256552.1	62.27
NEU1	Sialidosis, type I| Sialidosis, type II	AR|AR	NM_000434.3	119.58
NPHP4	Nephronophthisis 4| Senior-Loken syndrome 4	AR|AR	NM_015102.4	52.17
NPHS1	Nephrotic syndrome, type 1	AR	NM_004646.3	112.08
NPHS2	Nephrotic syndrome, type 2	AR	NM_001297575.1	86.29
NUP107	Galloway-Mowat syndrome 7; nephrotic syndrome, type 11; ?ovarian dysgenesis 6	AR	NM_020401.3	60.98
NUP205	?Nephrotic syndrome, type 13	-	NM_015135.2	71.10
NUP93	Nephrotic syndrome, type 12	AR	NM_014669.4	56.54
NXF5	-	-	NM_032946.2	83.26
OCRL	Dent disease 2| Lowe syndrome	XLR; |XLR	NM_001587.3	94.16
OSGEP	Galloway-Mowat syndrome 3	AR	NM_017807.3	62.15
PAX2	Glomerulosclerosis, focal segmental, 7; papillorenal syndrome	AD; AD	NM_001304569.1	106.34
PDSS2	Coenzyme Q10 deficiency, primary, 3	AR	NM_020381.3	119.97
PLCE1	Nephrotic syndrome, type 3	AR	NM_016341.3	124.63
PMM2	Congenital disorder of glycosylation, type Ia	AR	NM_000303.2	133.97
PODXL	-	-	NM_001018111.2	61.63
PTPRO	Nephrotic syndrome, type 6	AR	NM_030668.2	137.09
SCARB2	Epilepsy, progressive myoclonic 4, with or without renal failure	AR	NM_001204255.1	123.97
SGPL1	Nephrotic syndrome, type 14	AR	NM_003901.3	66.27
SMARCAL1	Schimkeimmunoosseous dysplasia	AR	NM_001127207.1	122.11
SPRY2	IgA nephropathy, susceptibility to, 3	AD	NM_001318536.1	99.38
SYNPO	-	-	NM_001166208.1	87.84
TP53RK	Galloway-Mowat syndrome 4	AR	NM_033550.3	62.52
TPRKB	Galloway-Mowat syndrome 5	AR	NM_001330386.1	64.71
TRPC6	Glomerulosclerosis, focal segmental, 2	AD	NM_004621.5	96.58
TTC21B	Nephronophthisis 12	AR,AD	NM_024753.4	142.42
TUBAL3	-	-	NM_001171864.1	61.15
VIPAS39	Arthrogryposis, renal dysfunction, and cholestasis 2	AR	NM_001193314.1	125.19

VPS33B	Arthrogryposis, renal dysfunction, and cholestasis 1	AR	NM_001289148.1	137.52
WDR73	Galloway-Mowat syndrome 1	AR	NM_032856.3	73.33
WT1	Denys-Drash syndrome, Frasier syndrome, nephrotic syndrome, type 4	AD,somaticmutation	NM_000378.4	103.30
XPO5	-	-	NM_020750.2	57.90
ZMPSTE24	Mandibuloacral dysplasia with type B lipodystrophy; restrictive dermopathy; lethal	AR	NM_005857.4	118.18

Supplementary Table SII Clinical and demographic characteristics of included patients

ID	*Sex*	*Religion*	*State of origin*	*Age at onset, days*	*Consanguinity*	*Family history*	*Extra-renal features*	*Low birth weight*	*Prematurity*	*Seizures*	*Weight SDS*	*Length SDS*	*eGFR, ml/min per 1.73 m²*	*Serum albumin, g/dl*	*Total cholesterol, mg/dl*
4^1	Boy	Hindu	Haryana	5	0	0	Developmental delay	0	1	0	-1.05	-3.68	86.67	2.2	492
8	Boy	Hindu	Uttar Pradesh	30	0	0	0	1	1	1	-1.56	-4.46	60.00	1.5	171
18	Boy	Muslim	Uttar Pradesh	15	1	0	Clubbing	1	1	1	0.2	NA	60.00	0.6	201
28	Boy	Hindu	Punjab	20	0	0	Atrial septal defect	0	0	0	NA	NA	65.00	3.1	NA
30	Girl	Hindu	Delhi	NA	0	0	0	0	0	0	NA	NA	NA	NA	NA
40^1	Boy	Hindu	Delhi	5	0	1	0	1	0	1	NA	NA	22.60	1	NA
46	Boy	Hindu	NK		0	0	0	0	0	0	NA	NA	NA	NA	NA
47^1	Boy	Hindu	Rajasthan	30	0	0	0	0	0	0	-2.48	-4.63	24.00	2.3	283
51	Boy	Hindu	Delhi	90	0	1	Oculocutaneous albinism, developmental delay, microcephaly, hepatomegaly	0	0	1	NA	NA	4.24	2.1	NA
52	Girl	Hindu	Delhi	NA	0	0	0	0	0	0	NA	NA	NA	NA	NA
59^3	Girl	Hindu	Nepal	45	0	1	Atrial septal defect	1	1	0	-1.88	-4.06	21.85	1.1	391
80	Boy	Hindu	Telangana	30	0	0	0	1	1	0	-3.94	NA	NA	1.2	188
85	Girl	Hindu	Madhya Pradesh	15	0	1	0	0	0	0	-3.29	-11.73	49.00	1.3	349
146^1	Girl	Hindu	Delhi	4	0	0	0	1	1	1	-4.02	-3.54	46.40	1.2	233
150^2	Boy	Hindu	Punjab	60	0	0	0	1	1	1	-3.14	-2.16	36.00	1.1	247
154	Girl	Muslim	Uttar Pradesh	4	1	1	Hiatus hernia, microcephaly, developmental delay, hypotonia	1	0	0	-2.2	-1.3	64.00	0.9	406
157	Boy	Hindu	Uttar Pradesh	15	0	1	Aqueductal stenosis,	0	0	1	-4.61	-5.12	58.00	1.3	295

							obstructive hydrocephalus								
162^[1]	Boy	Muslim	Delhi	30	1	0	0	1	1	0	0.6	NA	96.00	1.3	428
163	Girl	3	Punjab	30	0	0	0	0	0	0	-4.55	-3.92	77.33	1.4	234
165^[3]	Girl	Hindu	Bihar	45	0	0	0	0	0	0	-4.22	-2.85	32.00	0.9	276
168	Girl	Muslim	Uttar Pradesh	20	1	1	Café au lait spot; hirsutism	1	0	0	-2.73	-1.71	104.00	1.7	243
169^[2]	Girl	Muslim	Uttar Pradesh	26	1	0	0	1	0	0	-3.61	-4.07	94.00	0.6	402
173^[1,2]	Girl	Hindu	Uttar Pradesh	60	0	0	0	1	1	1	-4.14	-5.17	17.00	0.7	172
180	Girl	Hindu	Uttar Pradesh	85	0	0	0	0	1	0	-4.32	-6.39	200.00	0.8	250
181	Boy	Muslim	Uttar Pradesh	12	0	0	Dysmorphic facies	0	1	0	0.8	NA	36.00	1.2	342
196^[1]	Boy	Muslim	Uttar Pradesh	15	1	0	0	1	0	1	-4.14	-4.52	28.29	0.9	474
201^[1]	Girl	Muslim	Delhi	20	1	1	0	0	0	0	-2.23	-3.37	196.00	0.56	237
217	Boy	Muslim	Rajasthan	2	1	1	0	NA	NA		-2.69	-0.23	23.66	1.44	309
228^[1]	Boy	Hindu	Uttar Pradesh	15	0	0	0	NA	NA	1	-4.91	-0.5	201.00	1.1	239
235^[1]	Girl	Muslim	Uttar Pradesh	45	0	0	0	1	0	0	-5.83	-5.17	123.90	1.4	200
240	Boy	Hindu	Puducherry	70	1	1	Dysplastic ears	NA	NA	0	-0.12	-0.59	134.23	0.9	271
266	Boy	Muslim	Delhi	15	1	0	0	NA	NA	NA	NA	NA	NA	1.23	296
267^[3]	Girl	Hindu	Bihar	15	0	1	Atrial septal defect	0	0	0	NA	NA	NA	1.2	NA
X1	Girl	Muslim	Punjab	15	1	0	Microcoria,microcornea	NA	NA	NA	NA	NA	NA	NA	NA

eGFR estimated glomerular filtration rate; NA not available

^Indicates patients that shared the following variations: [1]c.2600G>A; [2]c.3478C>T and [3]c.C1099C>T

Supplementary Table SIII Quality metrics based on raw (FASTQ) and mapped (BAM) reads

Sample ID	*Total mapped reads*	*Percentage of total mapped reads over reference genome*	*Mean region coverage depth*
4	36903956	73.80%	48.2
8	36883757	74.50%	48.9
18	39101579	76.00%	52.8
28	39925438	97.86%	57.45
30	33396553	97.6%	43.96
40	36402136	97.77%	49.82
46	46559034	97.63%	57.5
47	48347870	97.67%	51.46
51	41882937	98.14%	58.09
52	54820473	97.81%	59.33
59	43196032	97.71%	58.23
80	46494263	97.72%	57
85	41809738	97.7%	42.99
146	35916462	97.55%	42.14
150	48251461	97.55%	49.67
154	37484353	98.19%	50.85
157	47974741	97.63%	51.34
162	50014242	97.72%	54.71
163	33651571	97.79%	44.01
165	48225036	97.75%	61.04
168	47661449	98.01%	51.14
169	35009940	97.71%	41.01
173	33637511	97.71%	34.08
180	28249162	97.89%	37.79
181	32296239	97.56%	34.09
196	30935558	97.36%	35.64
201	33626554	98.26%	59.42
217	74654485	98.17%	115.89
228	55633896	98.08%	90.73
235	66570469	98.09%	97.98
240	35311365	97.81%	53.55
266	40554026	97.94%	66.39
267	48404929	98.04%	82.57
X1	53300045	99.17%	90.9

Supplementary Table SIV Lists of prioritized variants for individual patients. Variants in bold were considered relevant

Patient ID	*Gene*	*Chromosome: Position*	*Exon*	*Consequence (Base-pair and amino acid)*	*Zygosity*	*Change*	*Frequency in 1KG; Exac; Exac_SAS; gnomAD*	*Polyphen*	*CADD-Phred*	*Eigen_raw*	*GERP++_RS*
4	***NPHS1***	**19:36317523**	**29**	**c.3619delG:p.E1207Kfs*29**	**Heterozygous**	**fs*del**	**.; .; .; $4.0x10^{-6}$**	**.**	**.**	**.**	**.**
	NPHS1	**19:36333089**	**19**	**c.G2600A:p.G867D**	**Heterozygous**	**NS**	**.; 0.00002173; 0.0001; 0.00001634**	**D**	**28.7**	**0.74**	**3.88**
	ITGB4	17:73753376	39	c.G5314A:p.E1772K	Heterozygous	NS	0.0002; 0.000059; 0; 0.000072	B	23	-0.305	3.03
8	***NPHS1***	**19: 36335078_36335079delGT**	**16**	**c.2138_2139delAC: p.D713Gfs*12**	**Heterozygous**	**fs*del**	**.; .; .; .**	**.**	**.**	**.**	**.**
	NPHS1	**19:36341342**	**5**	**c.C532T: p.Q178X**	**Heterozygous**	**Tr***	**.; .; .; 0.00001193**	**.**	**35**	**0.545**	**3.26**
	COQ8B	19:41198902	14	c.T1250C: p.L417P	Heterozygous	NS	.; .; .; .	D	25.6	0.831	5.37
18	***NPHS1***	**19:36322049**	**27**	**c.3388-1G>A**	**Homozygous**	**Sp**	**.; 0.0000083; 0; $3.98x10^{-6}$**	**.**	**22.2**	**0.771**	**4.1**
	NPHS1	19:36333098	19	c.G2591A: p.R864H	Homozygous	NS	0.0002; 0.0001; $7.2x10^{-5}$; 0.0001	D	34	0.501	4.93
	FN1	2:216242961	35	c.G5647C: p.V1883L	Heterozygous	NS	.; .; .; .	B	22.9	-0.123	4.6
28	***NPHS2***	**1:179530456**	**3**	**c.G419A: p.G140E**	**Homozygous**	**NS**	**.; $8.24 x10^{-6}$; $6.1 x10^{-5}$; .**	**D**	**32**	**0.983**	**5.82**
	MUC1	1:155162020	2	c.C113G: p.S38W	Heterozygous	NS	0.0004; 0.0002; 0.0013; 0.0002	D	22	-0.814	-3.48
30	No variants prioritized										
40	***NPHS1***	**19:36333089**	**19**	**c.G2600A: p.G867D**	**Homozygous**	**NS**	**.; 0.000022; 0.0001; 0.000016**	**D**	**28.7**	**0.74**	**3.88**
	ALMS1	2:73799812	16	c.A10805G: p.N3602S	Heterozygous	NS	.; 0.0001; 0.001; 0.0001	D	24.5	0.46	4.44
	ARHGDIA	17:79826497	7	c.G758A: p.R253H	Heterozygous	NS	.; .; .; $7.50 x10^{-6}$	.	.	.	.
46	***PLCE1***	**10:96058156**	**24**	**c.C5188T: p.Q1730X**	**Homozygous**	**Tr***	**.; .; .; .**	**.**	**41**	**0.964**	**5.6**
	ALMS1	2:73653592	6	c.G1249T: p.G417W	Heterozygous	NS	.; .; .; .	D	26	0.325	3.8

	INF2	14:105181131	21	c.G3632T: p.R1211L	Heterozygous	NS	.; 0.0002; 0.0011; 0.000098	P	22.8	-0.822	-2.53
47	***NPHS1***	**19:36333089**	**19**	**c.G2600A: p.G867D**	**Homozygous**	**NS**	**.; 0.000022; 0.0001; 0.00001634**	**D**	**28.7**	**0.74**	**3.88**
	COL4A3	2:228173699	49	c.G4547A: p.R1516Q	Heterozygous	NS	.; 0.000075; 0.0003; 0.000072	D	28.4	1.083	5.97
	CRB2	9:126128285	3	c.508_509del: p.C170fs	Heterozygous	fs*del	.; .; .; .	.	.	.	.
51	*COL4A4*	2:227924195	28	c.C2309T: p.P770L	Heterozygous	NS	.; 0.0000663; 0; 0.00003606	D	25.1	0.717	5.99
	NUP93	16:56864492	10	c.G980A: p.R327H	Heterozygous	NS	.; 0.0000165; 0.0001; 0.00002388	D	34	0.949	5.11
52	*ARHGAP24*	4:86921681	10	c.G2053A: p.D685N	Heterozygous	NS	0.0008; 0.0009; 0.0064; 0.0009	P	29.3	0.295	5.56
	SCARB2	4:77116941	2	c.A194G: p.Y65C	Heterozygous	NS	0.0006; 0.0003; 0.0021; 0.0003	D	25.3	0.53	4.35
59	***NPHS1***	**19:36339610**	**9**	**c.C1099T: p.R367C**	**Homozygous**	**NS**	**.; 0.00003308; 0.0001; 0.00003978**	**D**	**28.7**	**0.528**	**4.37**
	ARHGAP24	4:86921681	10	c.G2053A: p.D685N	Heterozygous	NS	0.0008; 0.0009; 0.0064; 0.0009	P	29.3	0.295	5.56
80	***NPHS1***	**19:36336350**	**14**	**c.A1850G: p.H617R**	**Heterozygous**	**NS**	**.; 0.0000084; 0; $7.98x10^{-6}$**	**D**	**22.8**	**0.43**	**4.56**
	NPHS1	**19:36340176**	**7**	**c.C802T: p.R268X**	**Heterozygous**	**Tr***	**.; 0.000034; $6.1x10^{-5}$; 0.00002407**	**.**	**35**	**0.166**	**2.65**
	COQ2	4:84188827	6	c.G1013A: p.G338E	Heterozygous	NS	.; .; .; 0.00001133	P	23.6	0.125	4.77
	CUBN	10:16918949	57	c.A9053C: p.Y3018S	Heterozygous	NS	.; 0.0001; 6.1 $x10^{-5}$; 0.0001	D	23.2	0.24	3.39
	TTC21B	2:166805994	3	c.C172T: p.R58X	Heterozygous	Tr*	.; 0.000058; 0.0002; 0.000040	.	36	0.751	4.52
85	***NPHS1***	**19:36339251**	**10**	**c.C1219T: p.R407W**	**Homozygous**	**NS**	**.; $8.2x10^{-6}$; 0; 0.000020**	**D**	**27**	**0.207**	**3.29**
	ANKS6	9:101558508	1	c.G266A: p.G89D	Homozygous	NS	.; .; .; 0	B	27.5	0.069	3.36
	KANK2	19:11304445	4	c.G311C: p.G104A	Heterozygous	NS	0.0004; 0.0005; 0.0037; 0.0004	D	13.59	-0.083	4.38
146	***NPHS1***	**19:36333089**	**19**	**c.G2600A: p.G867D**	**Homozygous**	**NS**	**.; 0.000022; 0.0001; 0.000016**	**D**	**28.7**	**0.74**	**3.88**
	KANK1	9:710861	7	c.T95C: p.F32S	Heterozygous	NS	0.0006; 0.0004; 0.0023; 0.0003	P	12.73	0.092	3.58

150	***NPHS1***	**19:36321958**	**27**	**c.C3478T: p.R1160X**	**Homozygous**	**Tr***	**.; 0.000066; 0.0002; 0.00009943**	**.**	**37**	**0.251**	**3.74**
154	***OSGEP***	**14:20920566**	**2**	**c.A157T: p.I53F**	**Homozygous**	**NS**	**.; $2.47x10^{-5}$; 0.000182; $1.22x10^{-5}$**	**B**	**15.82**	**-0.4**	**0.619**
	ANLN	7:36459872	11	c.G1964A: p.R655Q	Heterozygous	NS	.; 0.000016; 0.0001; $7.96 x10^{-6}$	P	29.3	0.455	4.95
	MAGI2	7:77649189	22	c.G3811A: p.A1271T	Heterozygous	NS	0.0004; 0.0002; 0.0012; 0.0002	P	28.3	-0.075	4.59
157	***NPHS1***	**19:36333370**	**18**	**c.C2417A: p.A806D**	**Homozygous**	**NS**	**.; $8.24 x10^{-6}$; 0; $7.95 x10^{-6}$**	**D**	**23.7**	**0.374**	**4.46**
	INF2	14:105181022	21	c.G3523A: p.D1175N	Heterozygous	NS	.; 0.000017; 0; $8.15 x10^{-5}$	D	19.12	0.097	4.73
	NPHP4	1:5937173	20	c.C2797T:p.R933W	Heterozygous	NS	.; 0.0000416; 0; 0.00002938	P	16.48	-1.152	-9.61
162	***NPHS1***	**19:36333089**	**19**	**c.G2600A:p.G867D**	**Homozygous**	**NS**	**.; 0.000022; 0.0001; 0.000016**	**D**	**28.7**	**0.74**	**3.88**
	ALMS1	2:73747129	11	c.C9764G:p.S3255C	Heterozygous	NS	0.001; 0.0006; 0.0044; 0.0005	D	23.4	-0.174	3.76
	COL4A1	13:110817289	46	c.G4070C:p.G1357A	Heterozygous	NS	.; .; .; .	D	26	0.667	4.3
	ITGB4	17:73739874	26	c.C3043T:p.R1015C	Heterozygous	NS	.; 0.0001; 0.0007; 0.0001	D	28.9	0.186	2.94
	KANK1	9:742265	14	c.C3757T:p.L1253F	Heterozygous	NS	.; .; .; $3.98 x10^{-6}$	D	26.3	0.472	4.28
163	***NPHS1***	**19:36335272**	**15**	**c.C2020T:p.P674S**	**Homozygous**	**NS**	**.; .; .; .**	**D**	**27**	**0.728**	**3.67**
	KANK1	9:710853	7	c.87delC: p.D29fs	Heterozygous	fs*del	.; $8.4x10^{-6}$; $6.8 x10^{-5}$; $3.99 x10^{-6}$	.	.	.	.
	VPS33B	15:91561079	2	c.C133G: p.L45V	Heterozygous	NS	0.0002; 0.000058; 0.0004; 0.000043	D	25.7	0.668	5.45
165	***NPHS1***	**19:36339610**	**9**	**c.C1099T: p.R367C**	**Heterozygous**	**NS**	**.; 0.00003308; 0.0001; 0.00003978**	**D**	**28.7**	**0.528**	**4.37**
	NPHS1	**19:36341349**	**5**	**c.527-2A>G**	**Heterozygous**	**Sp**	**.; .; .; .**	**.**	**22.6**	**0.788**	**4.09**
	ALMS1	2:73680160	8	c.C6503T: p.S2168L	Heterozygous	NS	0.0002; 0.000075; 0.0005; 0.000060	D	25.2	0.094	3.48
168	***NPHS1***	**19:36330221**	**22**	**c.C3027G: p.Y1009X**	**Homozygous**	**Tr***	**.; $8.24 x10^{-6}$; $6.1 x10^{-5}$; $7.95 x10^{-6}$**	**.**	**38**	**0.513**	**1.06**
	ALMS1	2:737997787	17	c.A10771C: p.T3591P	Heterozygous	NS	0.0008; 0.0003; 0.0025; 0.0003	D	18.17	-0.413	-1.18
	ARHGAP24	4:86921681	10	c.G2053A: p.D685N	Heterozygous	NS	0.0008; 0.0009; 0.0064; 0.0009	P	29.3	0.295	5.56

	CRB2	chr9:126135651 Exon-10	10	c.2841delG: p.P947fs	Heterozygous	fs*del	.; .; .; .	.	.	.	.
169	***NPHS1***	**19:36321958**	**27**	**c.C3478T: p.R1160X**	**Homozygous**	**Tr***	**.; 0.000066; 0.0002; 0.000099**	**.**	**37**	**0.251**	**3.74**
	CD151	11:837277	6	c.A379C: p.K127Q	Heterozygous	NS	0.0002; 0.0004; 0.0031; 0.0004	B	15.64	-0.087	4.26
	TTC21B	2:166747104	24	c.C3148T: p.R1050W	Heterozygous	NS	.; .; .; 3**.99** x10^{-6}	D	34	0.891	4.76
173	***NPHS1***	**19:36321958**	**27**	**c.C3478T: p.R1160X**	**Heterozygous**	**Tr***	**.; 0.000066; 0.0002; 0.000099**	**.**	**37**	**0.251**	**3.74**
	NPHS1	**19:36333089**	**19**	**c.G2600A: p.G867D**	Heterozygous	NS	.; 0.000022; 0.0001; 0.000016	D	28.7	0.74	3.88
	ITGA3	17:48153013	12	c.C1588T: p.R530C	Heterozygous	NS	.; 0.00002481; 6.1x10^{-5}; 0.00002389	D	27.8	0.244	5.54
	SYNPO	5:150036540	3	c.G2603A: p.G868E	Heterozygous	NS	0.0002; 0.0004; 0.0019; 0.0001	D	17.24	0.444	4.91
180	***NPHS1***	**19:36337056**	**12**	**c.C1481A: p.S494X**	**Heterozygous**	**Tr***	**.; .; .; .**	**.**	**39**	**0.581**	**4.15**
	NPHS1	**19:36340541-36340548**	**6**	**c.614_621delinsTT: p.T205_A207delinsI**	**Heterozygous**	**fs*del**	.; **0.00001653**; **0.0001; 0.0000199**	**.**	**.**	**.**	**.**
	NPHP4	1:5937221	20	c.2749delG:p.E917fs	Heterozygous	fs*del	.; .; .; .	.	.	.	.
181	***NPHS1***	**19:36332715**	**20**	**c.T2717C:p.I906T**	**Homozygous**	**NS**	**.; .; .; .**	**D**	**26.8**	**0.821**	**4.78**
	FN1	2:216271099	19	c.C2848T:p.H950Y	Heterozygous	NS	.; 8.24x10^{-6}; 6.1x10^{-5}; 0.00003183	P	24.6	0.271	5.14
196	***NPHS1***	**19:36333089**	**19**	**c.G2600A:p.G867D**	**Homozygous**	**NS**	**.; 0.00002173; 0.0001; 0.00001634**	**D**	**28.7**	**0.74**	**3.88**
	KANK2	19:11304341	4	c.G415T:p.A139S	Heterozygous	NS	.; .; .; .	D	22.6	0.161	4.38
	NPHP4	1:5947516	18	c.G2315A:p.R772H	Heterozygous	NS	.; 0.00001685; 0; 8.06x10^{-6}	D	33	0.754	5.46
201	***NPHS1***	**19:36333089**	19	**c.G2600A:p.G867D**	**Homozygous**	NS	**.; 0.00002173; 0.0001; 0.00001634**	**D**	**28.7**	**0.74**	**3.88**
	EXT1	8:119122904	1	c.G382T:p.A128S	Heterozygous	NS	.; 0.00001648; 0.0001; 0.0000159	B	12.59	-0.582	5.47
	LRP2	2:170068592	37	c.C6166T:p.R2056W	Heterozygous	NS	0.0004; 0.0001; 0.001; 0.0001	D	34	0.815	5.88
217	*ARHGDIA*	17:79826519	7	c.T736G:p.C246G	Heterozygous	NS	.; .; .; .	.	10.02	1.931	0.929
	FAT1	4:187527277	17	c.G10297A:p.V3433I	Heterozygous	NS	0.0006; 0.0002; 0.0014; 0.0002	B	19.37	-0.266	5.56
	ITGB4	17:73727328	10	c.G1094A:p.R365Q	Heterozygous	NS	.; 0.0001; 0.0006; 0.0001	P	24.3	-0.129	4.12

228	***NPHS1***	**19:36333089**	19	**c.G2600A:p.G867D**	**Heterozygous**	**NS**	**.; 0.00002173; 0.00001634**	**0.0001;**	**D**	**28.7**	**0.74**	**3.88**
	NPHS1	**19:36340541-36340548**	6	**c.614_621delinsTT; p.T205_A207delinsI**	**Heterozygous**	**fs*del**	**.; 0.00001653; 0.0000199**	**0.0001;**	**.**	**.**	**.**	**.**
	COQ6	14:74427966	9	c.G907A:p.A303T	Heterozygous	NS	0.0002; 0.0003; 0.0003	0.0023;	B	24.1	0.071	5.33
	COQ9	16:57490845	5	c.A524G:p.K175R	Heterozygous	NS	0.0002; 0.0001; 0.0001	0.0009;	P	23.6	0.464	5.68
	ITGB4	17:73745039	27	c.C3229T:p.R1077C	Heterozygous	NS	.; 0.00002598; 0.00003209	0;	D	32	0.584	4.93
235	***NPHS1***	**19:36333089**	19	**c.G2600A:p.G867D**	**Homozygous**	**NS**	**.; 0.00002173; 0.00001634**	**0.0001;**	**D**	**28.7**	**0.74**	**3.88**
	LAMB2	3:49162269	21	c.A2974G:p.I992V	Heterozygous	NS	0.0004; 0.0005; 0.0005	0.0038;	D	23.9	0.562	4.36
240	*WT1*	11:32450063	2	c.T749A:p.M250K	Heterozygous	NS	0.0002; 0.0003; 0.0002	0.0019;	B	24.4	0.09	5.62
266	No prioritized variations											
267	***NPHS1***	**19:36339610**	9	**c.C1099T: p.R367C**	**Heterozygous**	**NS**	**.; 0.000033; 0.0000398**	**0.0001;**	**D**	**28.7**	**0.528**	**4.37**
	NPHS1	**19:36341889**	4	**c.C500T: p.P167L**	**Heterozygous**	**NS**	**.; $8.3x10^{-6}$; $6.1x10^{-5}$; $3.9x10^{-6}$**		**D**	**27.6**	**0.555**	**5.99**
	FAT1	4:187540958	10	c.C6782T: p.T2261M	Heterozygous	NS	0.001; 0.0009; 0.0008	0.0033;	D	24.6	0.624	5.05
X1	***LAMB2***	3:49168499		**c.C799T; p.R267Ter**	**Homozygous**	**Tr**	**-, 0, 0, -**		**D**	**36**	**0.85**	**4.76**

B benign; D deleterious; del deletion; fs frameshift; NS non synonymous; P pathogenic; Tr truncating

Supplementary Table SV List of 44 single nucleotide polymorphisms (SNP), flanking p.Gly867Asp, selected for haplotype analysis

Haplotype region	*Chromosomal Coordinates*	*SNP ID*	*Reference allele A*	*Alternate; allele B*	*P-value**
5'-H2	chr19:35850672	rs142125121	T	C	0.0268
	chr19:35863180	rs201159994	A	G	0.0005
	chr19:35863226	rs150552589	G	T	2.2498E-08
5'-H1	chr19:35898796	rs112270905	T	TC	7.0269E-11
	chr19:35898899	rs16970294	A	G	7.0269E-11
	chr19:35899037	rs113510419	T	C	7.0269E-11
	chr19:35899068	rs142160831	GTGA	G	7.0269E-11
	chr19:35991373	rs2293695	C	T	0.025
	chr19:35998362	rs4254439	T	G	0.0459
	chr19:36004106	rs4806163	A	G	1.5754E-06
	chr19:36004171	rs12460932	C	A	0.0459
	chr19:36017928	rs10775583	G	C	0.0005
	chr19:36018272	rs12461911	C	T	0.0001
	chr19:36033460	rs2239945	C	T	0.0001
5'-H	chr19:36048741	rs2230181	G	T	0.0152
	chr19:36157740	rs61741212	C	T	0.0332
	chr19:36168914	rs2285421	T	C	2.0518E-05
	chr19:36218478	rs11670414	C	T	0.0047
	chr19:36224705	rs231591	A	G	5.3275E-08
	chr19:36233470	rs3746278	G	A	0.0113
	chr19:36234489	rs28656784	T	C	0.0332
	chr19:36235431	rs3761087	A	G	0.0256
	chr19:36236909	rs10402601	G	C	0.0256
	chr19:36246418	rs11549030	C	G	0.0429
	chr19:36269915	rs231230	T	C	0.0081
	chr19:36270052	rs231231	A	G	0.0081
	chr19:36273534	rs2291067	G	A	0.0035
	chr19:36275074	rs62112163	G	A	0.0035
	chr19:36278470	rs231235	C	G	0.0081

	chr19:36321910	rs731934	G	A	0.001
	chr19:36322270	rs2071327	C	T	0.0007
	chr19:36322509	rs466452	G	A	1.0815E-05
Mutation	chr19:36333089	G867D	C	T	3.53E-16
3'-H1	chr19:36351935	rs35854130	G	T	0.00006686
	chr19:36549684	rs61742664	G	A	0.015
3'-H2	chr19:36574063	rs45567532	C	T	7.6486E-08
	chr19:36577579	rs4806263	C	T	7.6486E-08
	chr19:36577742	rs77938609	G	A	0.015
	chr19:36583651	rs61494900	G	A	7.6486E-08
	chr19:36590329	rs2285745	T	C	0.0015
	chr19:36594063	rs17851502	C	T	7.6486E-08
	chr19:36595436	rs1008328	A	C	0.0044
	chr19:36603703	rs2072605	T	A	0.0005
	chr19:36674305	rs4805162	A	G	0.0066
	chr19:36727365	rs2070132	G	A	0.0013

Supplementary Table SVI Haplotype analysis of single nucleotide polymorphism markers flanking the mutation, indicating segregation of a core haplotype along with the Gly867Asp variant

No.	*~12 kbp region*	*~135 kbp region*	*Core-Haplotype (~500 kbp region)*			*~153 kbp region*	*Allele Count*
	rs142125121-rs150552589	*rs112270905-rs2239945*	*rs2230181-rs466452*		*rs35854130-rs61742664*	*rs45567532-rs2070132*	
	5'-H2	*5'-H1*	*5'-H*	***G867D***	*3'-H1*	*3'-H2*	
1	ABB	BBBBAAAABBB	AAAAAAAAAAAAAAAAAB	**B**	BA	BBABBBBBAB	13
2	ABB	BBBBAAAABBB	AAAAAAAAAAAAAAAAAB	**B**	BA	AAAABABBAB	1
3	AAA	BBBBAAAABBB	AAAAAAAAAAAAAAAAAB	**B**	BA	BBABBBBBAB	1
4	AAA	AAAABABABAA	AAAAAAAAAAAAAAAAAB	**B**	BA	AAAAAABABA	2
5	BAA	AAAAABAABBB	AAAAAAAAAAAAAAAAAB	A	BA	BBABBBBBAB	1
6	AAA	AAAABABABBB	AAAAAAAAAAAAAAAAAB	A	AA	AAAAAABAAA	1
7	BAA	AAAAAAAABBB	AAAAAAAAAAAAAAAAAB	A	AB	AABAAABAAB	1
	Other haplotypes						46

**Allele A refers to the major allele and B refers to the minor allele. In cases of Gly867Asp, B is a mutant allele. The grey shaded area refers to the mutant allele associated core haplotype*

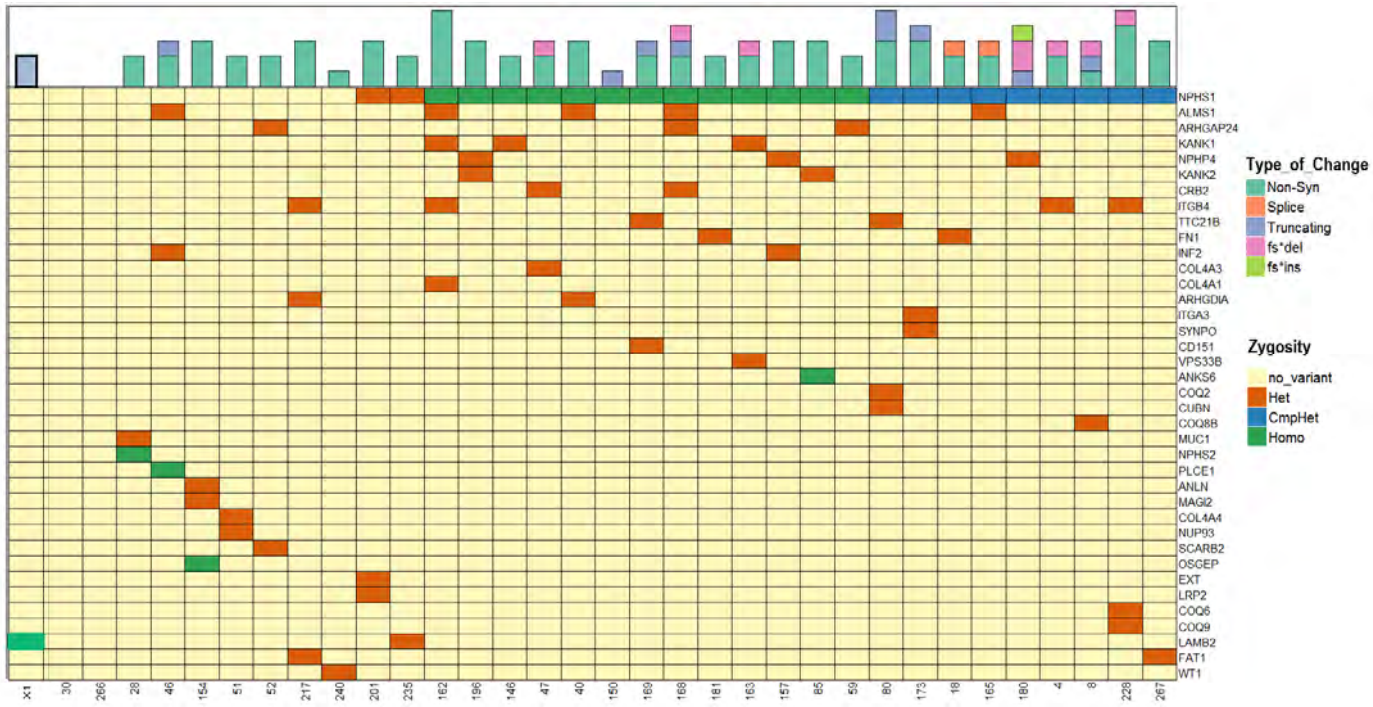
Supplementary SVII Studies examining the genetic basis of congenital nephrotic syndrome in 10 or more patients

Author, year	*N*	*Method of sequencing (number of genes)*	*Ethnicity*	*Etiology, %*						
				NPHS1	*NPHS2*	*WT1*	*PLCE1*	*LAMB2*	*Others*	*Unknown*
Koziell, 2002 [xi]	41	Sanger (2)	British, Maltese, Turkish, Asian	73	10	NT	NT	NT	NT	15
Sako, 2005 [xii]	13	Sanger (4)	Japanese	15	8	0	NT	NT	0 for *ACTN4*	77
Machuca, 2010 [xiii]	117	Sanger (8)	West Europe; Turkey; North Africa	61	15	2	2	2	0	19
Schoeb, 2010 [xiv]	67	Sanger (1)	Worldwide	58	Exc	Exc	NT	NT	NT	42
Buscher, 2010 [xv]	62	Sanger, panel (10)	German	53	13	23	2	5	2 (*ARGHDIA*)	3
Santin, 2011 [xvi]	15	Sanger (8)	Spanish	80	7	13	0	NT	NT	0
Lee, 2011 [xvii]	15	Not stated	Korean	40	7	40	0	7	0	7
Mbarek, 2011 [xviii]	12*	Linkage, Sanger (6)	Tunisian	60	0	0	0	40	0 for *CD2AP*	0
Cil, 2015 [xix]	80	Sanger (4)	Turkish; Middle East; East Europe	46	16	6	NT	4	NT	28
Sadowski, 2015 [xx]	235	Next-generation	Worldwide	40	11	9	2	6	3	31
Trautman, 2015 [xxi]	98	Sanger or next-generation	Europe, Middle East, Latin America	NA	NA	NA	NA	NA	NA	34
Sen, 2017 [xxii]	31	Next-generation	Worldwide	39	6	3	0	10	0	42
Wang, 2017 [xxiii]	12	Next-generation	Chinese	50	0	8	0	8	8 (*ADCK4*)	25
Li, 2018 [xxiv]	12	Sanger or next-generation	Chinese	67	0	8	0	0	8 (*COQ6*)	17
Sharief, 2019 [xxv]	11	Not stated	Arab, Asian, African	64	0	9	0	27	0	0
Nishi, 2019 [xxvi]	36	Not stated	Japanese	42	3	22	0	8	3 (*CRB2*)	22
Dufek, 2019 [xxvii]	69	Not stated	European	80	1	13	1	3	1 (*SGPL1*)	14
Berody, 2019 [xxviii]	55	Sanger (5)	European	65	9	7	2	0	0	16
Sinha, 2019 [xxix]	15	Next generation (27) or Sanger (<4)	Indian	53	0	7	7	0	0	33
Nagano, 2020 [xxx]	13	Targeted next generation	Japanese	31		15		31	8 (*LAMA5*)	15
Present study	34	Next-generation	Indian	74	4	0	4	4	4 (*OSGEP*)	11

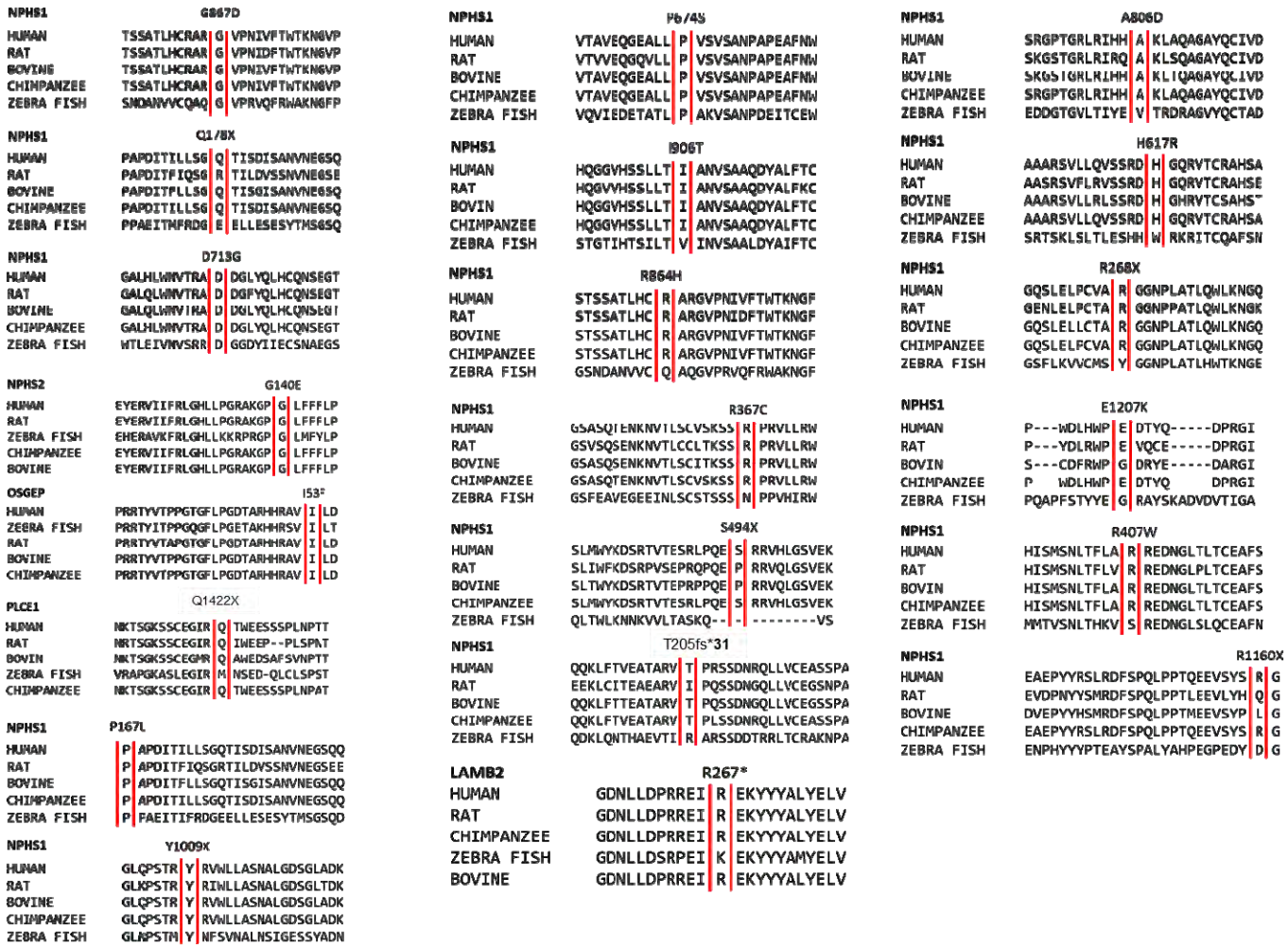
Exc excluded; NA not available; NT not tested
Only latest and largest paper for each group was included, unless overlap of patients between studies appeared unlikely
**Refers to 12 patients from 5 families*

Supplementary Table SVIII Frequency of haplotype markers of 5'-H1/5'H/3'-H1/3'-H2 region including Gly867Asp variant, (as indicated in Supplementary Table S6) from the1000 Genome population dataset

Sub-populations	*South Asian*	*European*	*East Asian*	*American*	*African*
Number of disease core haplotype carriers	2	20	1	7	1
Number of subjects	489	503	504	347	661
Frequency, %	0.20	1.98	0.09	1.008	0.07



Supplementary Figure S1 Heatmap representing prioritized variants per sample. Each column represents a patient while rows indicate genes. Individual cells are colored according to zygosity of variant while the type of change is indicated at the top of each column.



Supplementary Figure S2 Images indicating degree of conservation across species for variants to which pathogenicity was attributed

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REVIEW ARTICLE

Pediatric Coronavirus Disease 2019: Clinical Features and Management

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There is a lack of clarity regarding management of COVID-19 infection in children. This review aims to summarize the key clinical presentations and management of Pediatric COVID-19. The Medline database was searched for seminal articles and guidelines on COVID-19 presentation and management in children less than 18 years of age. COVID-19 has a lower incidence (1-5% of reported cases worldwide), causes milder disease with lower need for intensive care admission and lower mortality rate (0-0.7%) in children compared with adults. Multisystem inflammatory syndrome is a rare but severe complication in children. Majority of patients require supportive care including adequate hydration, nutrition and antipyretics. Supplemental oxygen therapy should be given in moderate to severe cases with all precautions to prevent air-borne COVID-19 spread. Steroids may be helpful in severe cases. Anticoagulation is indicated in moderate to severe cases with risk factors. More data on the efficacy and safety of antivirals and immunomodulators in children is needed.

Keywords: *Coronavirus, Dexamethasone, Remdesivir, SARS-CoV-2, Treatment*.

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The novel coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) on March 11, 2020. The causative agent, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), attaches through its viral surface spike proteins to the angiotensin converting enzyme-2 (ACE-2) receptors on the respiratory epithelial cells. Although several months into the pandemic, there is a lack of clarity regarding management of COVID-19 infection in children. This review aims to summarize the key clinical presentations and management of Pediatric COVID-19 based on most pertinent available evidence. The Medline database was searched for seminal articles on COVID-19 presentation and management in children less than 18 years of age. The latest guidelines from World Health Organization (WHO) and Ministry of Health and Family Welfare (MoHFW), Government of India were also reviewed [1,2].

EPIDEMIOLOGY IN CHILDREN

Children account for less than 5% of diagnosed COVID-19 infections worldwide [3]. As per the MoHFW, 8% of the COVID-19 positive cases in India were contributed by people below 17 years of age [4]. Reports show a lower need for hospital and intensive care unit (ICU) admission and lower mortality rate (0-0.7%) in children compared to adults [5]. This may be due to lower exposure, strong innate immune response due to trained immunity, healthier blood vessel endothelium, excellent alveolar epithelium regeneration capacity and fewer co-morbidities [6]. The community spread of virus by children is of concern as a high rate of asymptomatic infection is seen in younger age groups. However, data show lower transmission rate by children than adults [7,8].

CLINICAL FEATURES

The median age of presentation in children ranged from 3.3-11 years in different studies with a male preponderance [5,9,10]. When compared to adults, majority of COVID-19 infected children are asymptomatic with gastrointestinal and mild respiratory manifestations being the commonest [5,9-11]. Anosmia and ageusia are difficulty to elicit in young children and reported less commonly [5]. Other symptoms include lethargy, altered sensorium, seizures, sore throat, fatigue, myalgias, oligo-anuria, and skin rash. Severe or critical disease (acute respiratory distress syndrome, respiratory failure, shock, myocardial failure, and multiorgan dysfunction) is described in less than 1-3% children [10]. Viral co-infections have been reported in around 6% patients. Underlying co-morbidities (underlying malignancy, nephrotic syndrome, chronic disease of kidney, lung, or liver) are associated in 9.9 - 42% of SARS-CoV-2 positive children [12,13]. It is imperative to evaluate and treat these common infections and co-morbidities as COVID-19 may just be a bystander.

The COVID-19 disease severity classification is presented in **Table I** [1,14]. Indications for admission include children with moderate, severe or critical COVID-

Table I COVID-19 Disease Severity Classification

Clinical Severity	*Clinical presentation*	*Clinical parameters*
Mild	Symptomatic patients meeting the case definition for COVID-19	Fever, cough, sore throat, fatigue, anorexia, nasal congestion, malaise, headache, diarrhoea vomiting, nausea *without* evidence of viral pneumonia or hypoxia.
Moderate	Pneumonia	Child with clinical signs of pneumonia (cough or difficulty breathing *and* fast breathing *and/or* chest indrawing) *and* no signs of severe pneumonia. Fast breathing (in breaths/min): < 2 months: ≥60; 2-11 months: ≥50; 1-5 years: ≥40; 5-10 years: ≥30; 11-18 years: ≥24Chest imaging (radiograph, CT scan, ultrasound) may assist in diagnosis and identify or exclude pulmonary complications.
Severe	Severe pneumonia	Child with clinical signs of pneumonia at least one of the following: 1. Central cyanosis or SpO2 < 90% 2. Severe respiratory distress (e.g. grunting, very severe chest indrawing) 3. Any of the general danger signs: inability to breastfeed or drink, lethargy or unconsciousness, or convulsions.Chest imaging may provide corroborative evidence and identify or exclude complications.
Critical	Pediatric Acute Respiratory distress syndrome (PARDS) [14]	PARDS is said to occur in child with *all* of the following: 1. Acute onset (within 7 days of known clinical insult) 2. Respiratory failure (not fully explained by cardiac failure or fluid overload) with 3. Chest imaging findings of new infiltrate consistent with acute parenchymal disease with 4. Exclusion of perinatal related lung disease with 5. Oxygenation requirement as (a) or (b) (a) Non-invasive mechanical ventilation: Full face mask bi-level ventilation *or* CPAP ≥5 cm H_2O with PaO2: FiO2 ratio ≤300 *or* SpO2:FiO2 ratio ≤264 (b) Invasive mechanical ventilation: Mild: 4 ≤OI <8 *or* 5 ≤OSI <7.5) Moderate: 8 ≤OI <16 *or* 7.5 ≤OSI <12.3; Severe: OI ≥16 *or* OSI ≤12.3
	PARDS at risk [14]	PARDS at risk is said to occur in child with all of the above points 1 to 4 with oxygenation requirement as (a) or (b) (a) Non-invasive mechanical ventilation: Nasal mask CPAP or BiPAP requiring FiO2 ≤40% to attain SpO2 of 88-97%; or, Oxygen via mask, nasal cannula or high flow: SpO2 88-97% with oxygen supplementation at minimal flow as age <1 year: 2 L/min; 1-5 years: 4 L/min; 5-10 years: 6 L/min; >10 years: 8 L/min (b) Invasive mechanical ventilation: Oxygen supplementation to maintain SpO2 ≥88%, but OI< 4 *or* OSI <5
	Sepsis	*Suspected or proven infection and ≥2 of 4 age-based systemic inflammatory response syndrome (SIRS) criteria of which one must be (a) or (b)* a) Abnormal temperature (> 38.5 °C or < 36 °C) b) Abnormal white blood cell count for age or > 10% bands c) Tachycardia for age or bradycardia for age if < 1 year d) Tachypnoea for age or need for mechanical ventilation
	Septic shock	Any hypotension (SBP < 5th centile or > 2 SD below normal for age) corroborating with clinical markers *or*More than two of the following: a) altered mental status b) bradycardia or tachycardia (HR < 90 bpm or > 160 bpm in infants and h eart rate < 70 bpmor > 150 bpm in children) c) prolonged capillary refill (> 2 sec) or weak pulse d) mottled or cool peripheries e) reduced urine output

OI (Oxygenation index) = (FiO2 × mean airway pressure × 100)/Pao2; OSI (Oxygen saturation index) = (Fio2 × mean airway pressure × 100)/SpO2

19 disease. Mild disease can be managed at home. However, if the child has any underlying co-morbidity or if home isolation is not feasible, the child may managed at a COVID care centre or hospital.

INVESTIGATIONS

All patients with moderate to severe COVID-19 should undergo investigations as detailed in **Box I.** Investigations to rule out other possible differentials (like enteric fever, dengue, malaria, etc.) should be done as indicated.

MANAGEMENT

Mild Cases

Mild cases should be isolated at home, a community facility (COVID care-center) or a health facility decided on a case-to-case basis [1,2]. Pre-requisites for home isolation include apt residential conditions for quarantine of patient and family contacts, absence of co-morbidities and presence of a caregiver with communication link to the hospital. Strict adherence to home quarantine guidelines is necessary [22]. Any difficulty in breathing, grunting, inability to breast feed, bluish discoloration of lips or face, dip in oxygen saturation <95%, chest pain, mental confusion, inability to arouse and reduced interaction when awake should prompt urgent referral to a dedicated COVID health center or hospital. Symptomatic treatment should be given with antipyretic (Paracetamol) for fever and pain when necessary, adequate nutrition and rehydration, and identification and treatment of any underlying co-morbidities or co-infections. In children with symptomatic respiratory tract infection, routine use of antibiotics is not recommended except in situations of suspected or confirmed bacterial co-infection. Respiratory tract infection management should be followed as per existing protocols [23].

Asymptomatic cases who are incidentally detected like contacts of a diagnosed case or planned for an elective surgery may be isolated and monitored. A COVID positive status during surgery may pose a risk for infection spread and portend poor surgical outcome [24]. Therefore, elective surgeries should be delayed until patients test negative for COVID-19 [25,26].

Moderate Cases

Moderate cases should be treated in a dedicated COVID health center or hospital with detailed clinical history and regular assessment for vital signs, work of breathing and oxygen saturation (SpO2). Investigations as described in

Box I Suggested Investigations and Typical Findings in Children With Moderate to Severe COVID-19

Required	
Complete blood count (CBC):	Leucopenia has been reported in 6-26.3% children and lymphopenia in 3.5-40% children [15].
Liver function tests (LFT):	Persistent leucopenia, lymphopenia, and thrombocytopenia suggest severe disease.
Kidney Function test (KFT):	Raised liver transaminases may be seen in one-third children.
Chest *X*-ray (CT Thorax is best avoided for diagnostic screening routinely):	Milder and more focal findings than adults, typically as ground-glass opacities and consolidations with unilateral lower-lobe and peripheral predominance, which regress during recovery time [16].
Preferred	
D-dimer, coagulation profile, serum ferritin, C-reactive protein	Raised D-dimer and fibrinogen degradation products (found in 12-17.5% children), ferritin and CRP (found in 13.6% cases) levels are associated with severe disease, cytokine storm and multi-organ dysfunction [17]. Prolongation of aPTT and INR may be seen.
Electrocardiogram (ECG), Echocardiography (ECHO), Cardiac biomarker levels (troponin, CK and CK MB)	Prolonged PR interval, ST-T segment changes, atrioventricular block, arrhythmia, tachycardia, and low voltage are suggestive of cardiac injury, dysfunction in severe COVID-19. Raised cardiac enzymes indicate myocarditis or myocardial injury; are associated with severe disease ECHO may identify myocarditis, valvulitis, pericardial effusion, and coronary artery dilatation [18].
Desired	
LDH levels, serum IL-6 serum procalcitonin	Higher IL-6 and LDH levels are shown to correlate with disease severity but not routinely recommended [19,20]. Raised procalcitonin levels may indicate a bacterial co-infection or severe COVID-19 disease while a lower level has a high negative predictive value for a bacterial co-infection [21].

CT-computerized tomography; CK-creatine kinase; CK-MB-creatine kinase myocardial band; IL-6-interleukin-6; LDH-Lactate dehydrogenase.

Table II should be done at admission [1,2]. General management should be done as stated above. Additionally, the following may be considered [1,2].

i) Supplemental oxygen therapy should be used for distressed breathing or hypoxia (detailed below with management of severe cases). Bronchodilators if required are preferably delivered with an MDI and spacer instead of a nebulizer.

ii) Empiric antibiotic therapy may be given in under-five children. In the absence of hypoxia, an oral antibiotic (amoxycillin-clavulanic acid/azithromycin) may be added while intravenous ceftriaxone (50-100 mg/kg/day in two divided doses) may be started for moderate COVID-19 cases with hypoxia or infiltrates on chest X-ray.

iii) Chloroquine (5–10 mg/kg/day for 5-10 days) was used in children with moderate to severe COVID disease in initial months of the pandemic [27]. However, latest evidence shows no role of chloroquine or hydroxychloroquine in treatment of COVID-19 [28].

Close monitoring for disease progression, repeat investigations at 48-72 hours if needed and provision of transportation to dedicated COVID care hospital should be available.

Severe and Critical Cases

All severe and critical COVID-19 cases should be admitted in a dedicated COVID care hospital with detailed work-up as elucidated above. Continuous monitoring of vitals, work of breathing and SpO2 should be done.

i) All patients should be started on empirical intravenous antibiotics (third generation cephalosporins) within an hour of arrival which should be escalated as per clinical assessment.

ii) Aggressive intravenous fluid resuscitation should be avoided as it may worsen oxygenation.

iii) Experience of awake proning in children is limited as their tolerance may not be good and any agitation can worsen hypoxia.

iv) Supplemental oxygen therapy is required to maintain SpO2 ≥ 94% while taking all precautions to minimize aerosol generation.

The following modes of oxygen delivery may be used:

Conventional oxygen therapy may be given using nasal prongs/cannula, oxygen mask or hood. Non-rebreathing mask can provide up to 95% FiO2 at oxygen flow rate of 10 -15 L/ min and can be used for short periods initially [29].

HHHFNC/HFNC (Heated humidified high flow nasal canula**)** [30], is indicated in patients with mild ARDS without evidence of hemodynamic instability, altered mental status or multi-organ failure. However, in absence of response, consider early escalation to BiPAP/invasive ventilation. Although, increased aerosolization risk with HHHFNC has been speculated, the certainty of evidence is low and it is a widely preferred option in resource poor settings. A triple layer mask may be used to cover the mouth and nose of the patient over the nasal cannula to decrease aerosolization [29].

Non-invasive Ventilation

BiPAP (*Bilevel Positive Airway Pressure*): It is indicated for mild acute respiratory distress syndrome without hemodynamic instability, altered mental status or multi-organ failure. However, its use is feasible only in an older, cooperative child accepting of oronasal BiPAP mask [29].

Bubble CPAP (*Continuous positive airway pressure*) may also be used for newborns and children with severe hypoxemia.

Invasive Ventilation

Tracheal intubation should be performed when failure/ contraindication of BiPAP/HFNC occurs. The following specific precautions are needed:

- Pre-oxygenate with a non-rebreathing mask (NRM) or tight-fitting face mask attached to a self-inflating bag with100% oxygen for 5 minutes. Avoid bag and mask ventilation (BMV) to limit aerosolization and if required, use low tidal volumes.
- Follow Rapid sequence intubation using sedation and analgesia (to avoid cough reflex).
- Use a cuffed endotracheal tubes (ETT)
- Ensure intubation by most experienced person to minimize attempts and use video laryngoscope for intubation to maintain safe distance from patient.
- May use a plastic sheet to cover the head, neck and chest of patient to minimize contamination.
- Use disposable ventilator circuits and hydrophobic viral filter between the ventilator circuit at the expiratory end.
- Use closed suction to minimize contact with secretion and aerosol release.

The pediatric ARDS protocol for management should be used. Prone ventilation may be difficult to conduct in a child and may unnecessarily increase the risk of infection to the healthcare workers.

Extracorporeal membrane oxygenation (ECMO) may be considered in patients with continued severe hypoxemia despite maximal ventilatory support.

Management of Shock

Standard care includes early recognition and the initiation of antimicrobial therapy and slow crystalloid fluid bolus within 1 hour of recognition and vasopressors for fluid non-responsive hypotension. Further management may be as per the Surviving Sepsis Campaign guidelines for the management of septic shock in children [31].

Adjunctive Therapies for COVID-19

Steroids: Glucocorticoids may be considered for patients with severe or critical COVID-19 disease with progressive deterioration of oxygenation indicators, rapid worsening on imaging and excessive activation of the body's inflammatory response. The recommended doses include intravenous methylprednisolone 1 – 2mg/kg/day (maximum 80 mg) for 10 days or oral/ injectable dexamethasone 0.2-0.4 mg/kg/day OD (maximum of 6 mg) for 5 days [1,2]. These recommendations have been extrapolated from studies conducted chiefly in adults. The UK-based RECOVERY trial) reported dexamethasone to reduce mortality in patients who required respiratory support [32]. The proportion of children enrolled and analysed was not clear.

Anticoagulation [17,33]: Recommendations for use in children are listed in **Box II**. Thromboprophylaxis, both mechanical (with sequential compression devices, where feasible) and anticoagulation are recommended. Low molecular weight heparin (enoxaparin) 1.5 IU/kg/dose subcutaneous twice a day for <2 months age and 1 IU/kg/ dose twice a day for >2 months should be used. Unfractionated heparin may be used for children who are clinically unstable or have severe renal impairment as loading dose 75-100IU/kg intravenous in 10 min followed by initial maintenance dose of 28 IU/kg/hour for age <1 year and 20 IU/kg/hour for 1-18 years (target aPTT between 65-80 seconds). Anticoagulation therapy may be continued till resolution of the hypercoagulable state or resolution of the clinical risk factors for venous thrombo-embolism [17].

Thromboprophylaxis is contraindicated in active/ major bleeding, need for emergency surgery, platelets < 20,000/mm^3, concomitant aspirin administration at doses >5 mg/kg/d and malignant hypertension.

Remdesivir: There are no comparative clinical data evaluating the efficacy or safety of remdesivir for COVID-19 in pediatric patients. Although, initial guidelines contraindicated its use in children < 12 years, the US Food and Drug Administration issued an Emergency Use Authorization (EUA) to permit the use of remdesivir for treatment of COVID-19 in hospitalized pediatric patients [34]. As per NIH guidelines, remdesivir is indicated only in moderate COVID-19 with supplemental oxygen requirement where it shortens the time to recovery [34,35]. It may be considered in severe to critical COVID-19 (high flow oxygen device, NIV, invasive ventilation or ECMO) with dexamethasone (expert opinion) [34]. The latest guidelines are similar for children albeit extrapolated from adult data and recommended as a part of clinical trials [36]. Few case series in children show promise [37].

For children weighing > 40 kg, a single loading dose of 200 mg on day 1 followed by once daily dose of 100 mg from day 2 for 5-10 days is used. For children weighing 3.5-40 kg, a single loading dose of 5 mg/kg on day 1 followed by 2.5 mg/kg once daily from day 2 for 5-10 days may be given. The contraindications for its use include AST/ALT > 5 times upper limit of normal (ULN) and severe renal impairment (eGFR <30mL/min/m^2 or need for hemodialysis). Remdesivir should not be used in combination with chloroquine or hydroxychloroquine [34].

Tocilizumab (TCZ): It is a monoclonal antibody against interleukin-6 (IL-6) receptor which emerged as an alternative treatment for COVID-19 patients with cytokine

Box II Recommendations for Use of Thromboprophylaxis in Children With COVID-19

Moderate to severe COVID with any one of the following:

- D-Dimer levels more than 5 times the upper limit of normal
- One or more non-COVID risk factor for hospital acquired thrombo-embolism
- (Central venous catheter, mechanical ventilation, prolonged length of stay, complete immobility, obesity, active malignancy, nephrotic syndrome, cystic fibrosis exacerbation, sickle cell disease vaso occlusive crisis, flare of underlying inflammatory disease, congenital or acquired cardiac disease with venous stasis, previous history of VTE, First degree family history of VTE before age 40 years or unprovoked VTE, known thrombophilia, pubertal, post pubertal, or age >12 years, receiving estrogen containing oral contraceptive pill or post splenectomy for underlying hemoglobinopathy)
- Children with MISC with coronary artery aneurysms or low left ventricular ejection fraction

VTE: Venous thromboembolism; MISC: multisystem inflammatory syndrome in children.

storm. While initial systematic reviews show that TCZ resulted in reduction of mortality in severe COVID-19 cases compared to the standard treatment, the latest trials showed no benefit [38-40]. A larger ongoing RCT which is also enrolling children may provide clearer answers [41]. The use of TCZ is suggested only in context of clinical trials [34] in those with moderate/severe disease where oxygen/ventilation requirement is increasing after use of steroids with extensive bilateral lung disease on radio-imaging [2,17]. The dose of TCZ for >30 kg is 8 mg/kg (up to maximum of 800 mg) and <30 kg is 12 mg/kg given as intravenous infusion over 1 hour once, may be repeated if required at 12-24 hrs.

Contraindications to use include patients with HIV, those with active infections (uncontrolled systemic bacterial/fungal), tuberculosis, active hepatitis (total bilirubin or AST/ALT raised > 5 times ULN), ANC < 500-2000/mm3 and platelet count <50,000-1,00,000/mm3. Recipients should be carefully monitored for secondary infections, neutropenia, and thrombocytopenia. All patients should obtain a latent tuberculosis (TB) test before TCZ therapy. If the text is positive, treatment for tuberculosis should be started prior to administration although, the risk for latent TB reactivation is very compared to the benefit of administering TCZ. Safety profile of TCZ in COVID-19 patients is yet to be understood.

Convalescent plasma therapy (CPT): It may be considered in patients with moderate disease who are not improving with steroids. Few reports of its use in children with severe COVID-19 show promise [42,43]. Special considerations while using CPT include ABO compatibility, neutralizing titre of donor plasma above the specific threshold and avoidance of use in patients with IgA deficiency or immunoglobulin allergy. While adult trials have used doses of 4 to 13 ml/kg (usually 200 mL single dose) given slowly over 2 hours, 2-4 mL/kg of convalescent plasma has been used in children [43].

Other Agents Under Evaluation

Ivermectin, a potent in vitro inhibitor of the COVID-19 causative virus (SARS-CoV-2) with an established safety profile for human use, was shown to be beneficial in COVID-19 [44]. A newer agent under evaluation is the interleukin (IL)-1 inhibitor anakinra which may be considered for immunomodulatory therapy (>4 mg/kg/day intravenous or subcutaneous) in COVID-19 with hyperinflammation. Initiation of anakinra before invasive mechanical ventilation may be beneficial [33]. Other potential treatments under evaluation include interferon-beta, anti-IL-6 receptor monoclonal antibodies (sarilumab), anti-IL-6 monoclonal antibody (siltuximab), Bruton's tyrosine kinase inhibitors, acalabrutinib, ibrutinib, zanubrutinib) and Janus kinase inhibitors (baricitinib, ruxolitinib, tofacitinib). A recent trial has shown benefit of baricitinib-remdesivir combination compared to remdesivir alone in reducing recovery time in COVID-19 patients [45]. However, there is insufficient data for recommending use of any of these agents in children except in the context of a clinical trial [34].

Discharge Criteria and Follow-Up

The patient with mild to moderate disease can be discharged after 10 days of symptom onset and no fever or oxygen requirement for three consecutive days with complete resolution of symptoms prior to discharge [48]. Negative RT-PCR before discharge is not required. Home quarantine for 7 days post-discharge is necessary. Patients with severe disease and immunocompromised states (like cancer transplant recipients and HIV) should have complete resolution of symptoms and negative RT-PCR test report prior to discharge [48].

MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN (MIS-C)

MIS-C is a post-infectious inflammatory response syndrome (characterized by high levels of pro-inflammatory cytokines CTNF, IL-6 and IL-1β) following SARS-CoV-2 infection. Various diagnostic criteria have been provided by WHO and CDC [33]. A tiered investigational approach is followed in patients without life-threatening manifestations, while work-up is done simultaneously for the sick children [33]. Patients may require additional investigations to rule out any co-infection/other cause of illness.

Children with life threatening manifestations should be admitted in PICU management. Children with acute COVID inflammation (RT-PCR positive) with symptoms like Kawasaki disease (KD) should receive intravenous immunoglobulin (IVIG) (dose-2g/kg over 1-2 days) and remdesivir if available. Children with remote COVID infection with KD symptom overlap should receive IVIG and aspirin (20–25 mg/kg/dose every 6 hourly or 80-100 mg/kg/day) steroids may be added. In children with remote COVID infection with predominant cardiovascular involvement (myocarditis/cardiogenic shock/distributive shock) with or without KD symptom overlap IVIG, 3-day pulse of methylprednisolone with tapering and LMWH prophylaxis are to be considered as disease modifying agents [46].

Sick children should receive initial broad-spectrum antibiotics considering symptom overlap with severe bacterial infection. Ceftriaxone or meropenem with vancomycin or clindamycin or teicoplanin may be used

for the sickest children. In stable patients with MIS overlap, with mild lab abnormalities and lacking alternate diagnosis, ceftriaxone may be given. Metronidazole is added if gastro-intestinal symptoms are predominant.

All children with MIS-C require ongoing clinical monitoring while laboratory investigations may be repeated every 24-48 hourly as guided by the clinical condition [47].

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Steroid Sensitive Nephrotic Syndrome: Revised Guidelines

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Justification: Steroid sensitive nephrotic syndrome (SSNS) is one of the most common chronic kidney diseases in children. These guidelines update the existing Indian Society of Pediatric Nephrology recommendations on its management. **Objective**: To frame revised guidelines on diagnosis, evaluation, management and supportive care of patients with the illness. **Process**: The guidelines combine evidence-based recommendations and expert opinion. Formulation of key questions was followed by review of literature and evaluation of evidence by experts in two face-to-face meetings. **Recommendations**: The initial statements provide advice for evaluation at onset and follow up and indications for kidney biopsy. Subsequent statements provide recommendations for management of the first episode of illness and of disease relapses. Recommendations on the use of immunosuppressive strategies in patients with frequent relapses and steroid dependence are accompanied by suggestions for step-wise approach and plan of monitoring. Guidance is also provided regarding the management of common complications including edema, hypovolemia and serious infections. Advice on immunization and transition of care is given. The revised guideline is intended to improve the management and outcomes of patients with SSNS, and provide directions for future research.

Keywords: *Calcineurin inhibitors, Frequent relapses, Levamisole, Minimal change nephrotic syndrome, Mycophenolate mofetil, Rituximab, Steroid dependence.*

Nephrotic syndrome, characterized by edema, heavy proteinuria (>1 g/m^2 daily; >40 mg/m^2/hr) and hypoalbuminemia (serum albumin <3 g/dL), is among the most common kidney diseases in childhood. The condition has an annual incidence ranging from 1.2 to 16.9 per 100,000 children [1,2]. While nephrotic syndrome is usually primary or idiopathic, evaluation might reveal an underlying systemic illness in 5-10% of patients. Kidney biopsy reveals minimal change disease in ~80% patients, and focal segmental glomerulosclerosis (FSGS) and mesangioproliferative glomerulonephritis (GN) in 7-8% each. Therapy with prednisolone results in complete remission of proteinuria in 85-90% patients, termed steroid sensitive nephrotic syndrome (SSNS). While the outcome in patients with SSNS is satisfactory, approximately 50% show frequent relapses or steroid dependence, and 3-10% show late steroid resistance [3-5].

OBJECTIVE

Guidelines on management of SSNS, by the Indian Society of Pediatric Nephrology, were first published in 2001 [6] and updated in 2008 [7]. With increasing availability of evidence on various therapies, these guidelines have been revised. Guidance is based on the strength and quality of evidence using the GRADE model proposed by the American Academy of Pediatrics [8]. Ungraded statements (indicated by X) are like practice points, not supported by sufficient evidence. **Table I** highlights key changes in present guidelines compared to 2008 [7] and those recently proposed by the Kidney Disease Improving Global Outcomes [9].

PROCESS

Workgroups were constituted to address key issues, including: *(i)* Evaluation at baseline and follow up, role of biopsy, genetic testing, and differential diagnosis; *(ii)* Management of the initial episode and subsequent relapses; *(iii)* Management of frequent relapses; and *(iv)* Supportive care and outcomes. Separate workgroups have addressed guidelines on the definition and management of steroid resistant nephrotic syndrome [10]. The workgroups identified gaps in knowledge, formulated questions and developed consensus statements prior to the meeting in New Delhi on 5 April 2019, when the evidence was discussed through alternating breakout and plenary sessions. Research studies were rated from A to D using standard criteria, and

Table I Comparison Between Present and 2008 [7] Guidelines of the Indian Society of Pediatric Nephrology (ISPN), and Kidney Disease Improving Global Outcomes (KDIGO) 2021 [9]

Parameter	*ISPN 2021*	*ISPN 2008 [7]*	*KDIGO 2021 [9]*
Nephrotic syndrome	Nephrotic range proteinuria, hypoalbuminemia (albumin <3 g/dL) and edema	Nephrotic range proteinuria, hypoalbuminemia (<2.5 g/dL), cholesterol >200 mg/dL and edema	Nephrotic range proteinuria and either hypoalbuminemia (<3 g/dL) or edema
Steroid resistance	Lack of complete remission despite daily therapy with prednisolone for 6-wk	Lack of complete remission despite daily therapy with prednisolone for 4-wk	Lack of complete remission despite daily therapy with prednisone for 4-weeks[a]
Prednisolone for initial episode	6-wk daily and 6-wk AD; surface area (BSA) or weight-based dosing[b]; no indication for prolonged therapy	6-wk daily and 6-wk AD; weight-based dosing[b]; no indication for prolonged therapy	4-6 wk daily and 4-6 wk AD; BSA or weight-based dosing[b]; prolong therapy (16- 24 wk) if <4-6 yr-old, or if delayed remission
Frequent relapses	≥2 relapses in first 6-months after initial therapy; ≥3 relapses in any 6-mo; ≥4 relapses in 1 year	≥2 relapses in first 6-months after stopping initial therapy; ≥4 relapses in 1-year	≥2 relapses in 6-months; ≥4 relapses in 1-year
Prolonged AD prednisolone	Taper to 0.5-0.7 mg/kg AD for 6-12 months	Taper to 0.5-0.7 mg/kg AD, for 9-18 months	Limited role in view of risk of toxicity
Prednisolone during infections	Daily for 5-7 days, if receiving AD prednisolone	No recommendation	Daily at 0.5 mg/kg for 5-7 days, whether on/off steroids
Steroid sparing therapy: Indications, choice	Failure of AD therapy: Levamisole or MMF Steroid threshold >1 mg/kg AD, toxicity, complicated relapses: Cyclophosphamide, MMF Difficult-to-treat: CNI, then rituximab	Failure of AD therapy, steroid toxicity: Levamisole Steroid toxicity, severe relapses, poor compliance: Cyclophosphamide Failure of above therapies: CNI; MMF an option	Frequent relapses with steroid toxicity; patients with dependence Frequent relapses: Levamisole, cyclophosphamide Dependence: MMF, rituximab, cyclophosphamide, CNI
Supportive	Advice on diet, immunization, management of edema; calcium and vitamin D supplements		

AD-alternate days; CNI-calcineurin inhibitor; MMF-mycophenolate mofetil; [a]Late responder: Partial remission at 4 weeks and complete remission at 6 weeks of daily prednisone; [b]BSA-based dosing: 60 mg/m^2 daily and 40 mg/m^{2}AD; weight-based: 2 mg/kg/day and 1.5 mg/kg AD; maximum 60 mg daily and 40 mg AD.

each consensus statement was assigned one of two levels of recommendation, based on assessment of relative benefit versus harm, and relevance in context of availability and cost, and the feasibility of monitoring (**Supp. Table I**) [11]. Draft guidelines were again discussed in Pune on 21 December 2019. The final manuscript was circulated to all participants for approval.

DEFINITIONS

Criteria for defining the course of nephrotic syndrome are shown in **Box I** [12-14]. For purpose of this guidelines, unless stated, the term 'frequent relapses' includes patients with 'steroid dependence', and prednisolone and prednisone are used interchangeably. The management of initial and late resistance, defined as lack of remission following 6-weeks' prednisolone therapy (**Box I**) is discussed separately [10].

Patients with frequent relapsing and steroid resistant nephrotic syndrome are at high risk of complications, due to the illness and toxicity of medications. We advise that these patients, and those younger than one year, be managed by pediatric nephrologists.

Guideline 1: Evaluation

1.1 In a patient presenting with recent onset of edema, we recommend the following investigations to confirm the diagnosis of nephrotic syndrome: *(i)* urinalysis; and *(ii)* blood levels of urea, creatinine, albumin and total cholesterol (**Box II**). (X)

1.2 We suggest additional evaluation in selected patients (**Box II**). (X)

1.3 We recommend that parents be taught to maintain a record of proteinuria (by dipstick or boiling), infections and medications received. **(**X)

Rationale

Children with the first episode of nephrotic syndrome require evaluation to confirm the diagnosis and screen for

Box I Definitions of Disease Course and Severity in Nephrotic Syndrome

Nephrotic range proteinuria	Urine protein 3+ or 4+; urine protein to creatinine ratio (Up/Uc) >2 mg/mg in first morning urine specimen; proteinuria >40 mg/m^2/hr
Remission	Urine protein nil or trace (Up/Uc <0.2 mg/mg) for 3 consecutive early morning specimens
Relapse	Urine protein ≥3+ (Up/Uc >2 mg/mg) for 3 consecutive early morning specimens, having been in remission previously
Frequent relapses	Two or more relapses in the first 6-months after stopping initial therapy[a]; ≥3 relapses in any 6-months; or ≥4 relapses in one yr
Steroid dependence	Two consecutive relapses when on alternate day steroids, or within 14 days of its discontinuation
Steroid resistance[b]	Lack of complete remission despite therapy with daily prednisolone at a dose of 2 mg/kg (or 60 mg/m^2) daily for 6 weeks
Stable remission	Sustained remission or infrequent relapses during immunosuppressive therapy
Complicated relapse	Relapse associated with life-threatening complications: *(i)* hypovolemia requiring inpatient care, *(ii)* severe infection (peritonitis, cellulitis, meningitis), or *(iii)* thrombosis
Significant steroid toxicity	Hyperglycemia (fasting glucose >100 mg/dL, post-prandial glucose >140 mg/dL, or HbA1c >5.7%) [12]; obesity (body mass index >equivalent of 27kg/m^2 in adults [13]); short stature (height < –2 SDS for age [13]) with height velocity (< –3 SDS for age [14]); raised intraocular pressure; cataract(s); myopathy; osteonecrosis; or psychosis
Difficult-to-treat steroid sensitive disease	Both of the following: *(i)* frequent relapses, or significant steroid toxicity with infrequent relapses; and *(ii)* failure of ≥2 steroid sparing agents (including levamisole, cyclophosphamide, mycophenolate mofetil)

[a]Or during initial therapy; [b]Therapy in the last 2 weeks may be given on alternate days in patients with steroid toxicity. HbA1c-glycosylated hemoglobin; SDS-standard deviation score.

an underlying cause and complications. Family history of nephrotic syndrome, asthma and allergies, and renal diseases are asked for. Features including fever, abdominal pain, rash, arthralgia, oliguria, hematuria and history of drugs or infections suggest an underlying cause, e.g., systemic lupus erythematosus and IgA vasculitis. Height, weight and blood pressure should be recorded; weight monitoring helps in assessment for edema.

Investigations advised at the initial episode are listed in **Box II**. The diagnosis is based on presence of nephrotic range proteinuria, hypoalbuminemia and edema. Majority of patients show total cholesterol levels exceeding 200 mg/dL. Nephrotic range proteinuria is present if in an early morning urine sample protein is 3-4+ (dipstick/ boiling test), spot protein to creatinine ratio is >2 mg/mg, or the protein excretion is >40 mg/m^2 per hr. Precise estimation of 24-hr protein excretion is cumbersome, and is seldom necessary. Urine microscopy is normal, except for hyaline or granular casts; occasional microscopic hematuria is not uncommon. Persistent microscopic hematuria or red cell casts suggests disease other than minimal change nephrotic syndrome, like infection related GN, C3 glomerulopathy, systemic lupus or vasculitis [1]. Additional investigations are required for their diagnosis. Since patients with nephrotic syndrome do not have increased prevalence of urinary tract infections, routine urine cultures are not necessary.

With an estimated prevalence of bacteriologically positive pulmonary tuberculosis of 296 per 100,000 population in India, the risk of latent tuberculosis infection in childhood is high [15,16]. Tuberculin test is suggested prior to the first course of steroid treatment, especially with history of contact [16]. Chest radiography is done in patients with positive tuberculin test; those with features of tuberculosis require appropriate therapy. Patients with positive tuberculin reaction, but no radiological or bacteriological evidence of tuberculosis, should receive isoniazid prophylaxis for 6-months [16]. The prevalence of hepatitis B in non-tribal Indian populations is low (2.4%; 95% CI, 2.2-2.7%) [17], and routine screening is not required.

Genome wide association studies have identified variants in multiple MHC class II molecules as risk factors for SSNS [18]. The diagnostic and prognostic utility of various biomarkers of minimal change disease is limited [19]. There is, currently, no role for biomarkers or genetic studies in these patients.

Subsequent Evaluation

Parents are instructed to monitor the child's urine at home, using dipstick or boiling test, and are explained the features of a relapse. During remission, they are advised to screen for proteinuria 2-3 times a week; the child is also examined every day during infections, or if edema is present. Frequent assessment of biochemistry is not necessary. Evaluation of patients during relapses also includes screening for complications (**Box II**).

Guideline 2: Kidney biopsy

2.1 We recommend kidney biopsy in nephrotic syndrome,

in the presence of: *(i)* persistent microscopic hematuria, gross hematuria, or acute kidney injury not attributed to hypovolemia; *(ii)* systemic features: fever, rash, arthralgia, low complement C3; *(iii)* initial or late corticosteroid resistance; and *(iv)* prolonged (>30-36 months) therapy with calcineurin inhibitors (CNI), or reduced kidney function during their use. (1B)

2.2 We suggest performing kidney biopsy prior to initiating therapy with CNI. (X)

2.3 We recommend light microscopy and immunofluorescence examination on all kidney biopsies. Electron microscopy is required in patients with gross or persistent microscopic hematuria, low C3 and suspected disorders of glomerular basement membrane. (X)

Rationale

Clinicopathological studies show that kidney biopsy is not routinely required in children with idiopathic nephrotic syndrome prior to therapy with corticosteroids [20-22]. Remission of proteinuria following steroid therapy is the most important predictor of long-term outcome [3,23]. The chief indication of kidney biopsy is in patients who fail to show complete remission of proteinuria despite 6-weeks daily therapy with prednisolone (steroid resistant illness) [10,24]. A biopsy is indicated in patients with gross hematuria or persistent microscopic hematuria at the onset (> 5 red cells per high power field on 3 or more occasions, in urine centrifuged at 400 g for 4-5 minutes); or extrarenal features of a systemic disease [20-23,25].

Box II Investigations in Patients with Steroid Sensitive Nephrotic Syndrome

Essential at onset

Urinalysis[a]

Complete blood counts

Blood urea, creatinine, electrolytes, total protein, albumin, total cholesterol

Tuberculin test

Additional evaluation, at onset or relapse

Chest radiography: Positive tuberculin test or history of contact; suspected lower respiratory tract infection

Renal ultrasonography: Planned for kidney biopsy; presence of gross hematuria; suspected renal vein thrombosis

Complete blood counts: Suspected systemic infection or hypovolemia

Blood urea, creatinine, albumin, electrolytes: Severe edema; hypovolemia/dehydration; oliguria/anuria; prolonged (>72 h) diuretic therapy

Complement C3, C4, antinuclear antibody, antistreptolysin O: Gross, persistent microscopic hematuria; sustained hypertension; suspected secondary cause (systemic lupus, IgA vasculitis, C3 glomerulopathy)

Serum transaminases; hepatitis B surface antigen; antibody against hepatitis C virus: History of jaundice or liver disease

Periodic monitoring, if relapsing illness

Blood creatinine; albumin, electrolytes

[a]Quantitative estimation of urine protein is required if the diagnosis of nephrotic range proteinuria is uncertain.

An age of onset of more than 12-years is often cited as an indication for performing a kidney biopsy. Review of literature in adolescent onset nephrotic syndrome suggests that a combination of features, including persistent microscopic hematuria, low C3 and steroid resistance, detects all patients with membranous nephropathy or proliferative GN [20-22,26,27]. This might obviate the need for a kidney biopsy in adolescents presenting with typical nephrotic syndrome that is steroid sensitive. Since infants (<12-months-old), including those with congenital nephrotic syndrome, are likely to show histological features other than minimal change disease or an underlying genetic change, we advise next-generation sequencing in these patients [10]. Patients with onset of idiopathic nephrotic syndrome beyond infancy should receive therapy with prednisolone, and are advised to undergo kidney biopsy if they show steroid resistance.

The large majority of patients with SSNS show minimal change disease, and less commonly, FSGS or mesangioproliferative GN [20-22,28]. More than 90% children with minimal change disease, 50% with mesangioproliferative GN, and 30% with FSGS have steroid sensitive disease. Patients with frequent relapses do not require a biopsy before initiating therapy with steroid-sparing agents like levamisole, cyclo-phosphamide, mycophenolate mofetil (MMF) or rituximab [29]. The exception is prior to the use of CNI.

While there is limited guidance to support kidney biopsy in patients with SSNS prior to the therapy with CNI [9,30], information on the extent of tubular atrophy and interstitial fibrosis is useful when planning therapy. Therapy with CNI might result in acute nephrotoxicity, manifested as acute tubular injury and isometric tubular epithelial vacuolization [31,32]. Chronic nephrotoxicity, characterized by striped tubulointerstitial fibrosis has been reported in 25-43% biopsies following therapy (for 2.5-3.5 years) with cyclosporin or tacrolimus [33-35]. While a recent report found low risk of nephrotoxicity despite prolonged use of tacrolimus [36], most reports suggest similar risk with both medications [34,37]. We therefore suggest considering kidney biopsy before initiating therapy with CNI, particularly in patients with prolonged disease and unclear course, and to inform the clinician regarding baseline histological changes and

allow appropriate counseling. In view of long-term risks of nephrotoxicity, kidney biopsy should be performed following prolonged therapy with CNI, or if the therapy is associated with decline in eGFR that persists despite reduction in CNI dose [9,38].

An adequate biopsy specimen should preferably include the corticomedullary junction and approximately 20 glomeruli to exclude the diagnosis of FSGS [39]. Apart from renal histology, the biopsy provides information on extent and morphology of glomerulosclerosis and associated tubulointerstitial changes. The diagnosis of IgA nephropathy, C3 glomerulopathy and early membranous nephropathy is suggested by immunofluorescence studies. While kidney biopsies from all patients with nephrotic syndrome should be examined by electron microscopy, the facility is often not available. Ultrastructural examination helps to confirm the diagnosis of minimal change disease (effacement of podocyte foot processes; no electron dense deposits), differentiate primary from secondary FSGS (diffuse versus focal foot process effacement), categorize membranous nephropathy and C3 glomerulopathy, and identify disorders of glomerular basement membrane [40].

Guideline 3: Therapy for the first episode of nephrotic syndrome

We recommend that therapy for the initial episode should comprise of prednisolone at a dose of 60 mg/m^2/day (2 mg/kg/day, maximum 60 mg in 1-2 divided doses) for 6 weeks, followed by 40 mg/m^2 (1.5 mg/kg, maximum 40 mg as single morning dose) on alternate days for the next 6 weeks, and then discontinued. (1A)

Rationale

In 1981, the International Study of Kidney Disease in Children (ISKDC) proposed that the first episode of nephrotic syndrome be treated with daily prednisone for 4-weeks, followed by intermittent therapy for the next 4-weeks, and then discontinued [41]. Later, a randomized controlled trial (RCT) by the Arbeitsgemeinschaft für Padiatrische Nephrologie showed that therapy with prednisolone for 6-weeks daily and 6-weeks alternate-day was better in terms of reduced incidence of relapses over the next 12-24 months [42]. In efforts to define optimal therapy for the initial episode, several RCTs have investigated the duration and dose of prednisolone, based on which, a meta-analysis, in 2007, concluded that prolonging therapy for 6-months was associated with reduced risk of relapses and of frequent relapses (relative risk, RR 0.55; 95% CI 0.39-0.80) [43]. However, most studies included in this analysis had methodological flaws, resulting in a high risk of bias.

Four large multicenter RCTs published in the last 7 years have challenged the previous results (**Supp. Table II**). These studies, representing outcomes in over 800 patients across Netherlands, UK, Japan and India, show that extending initial therapy beyond 8-12 weeks does not influence either the time to first relapse or the risk of frequent relapses at 1-2 years' follow up. These studies had low risk of bias; three were placebo-controlled. A meta-analysis that included three of these studies, showed that the risk of frequent relapses at 1-2 years' follow-up was lower for 3-months or longer versus 2-months therapy (RR 0.68; 95% CI 0.47-1.0), but not for 5-months or longer versus 3-months therapy (RR 0.78; 95% CI 0.50-1.22) [44]. Subgroup analysis, limited to studies at low risk of bias, indicated similar risk for frequent relapses in patients treated for 2-3 months versus 3-6 months. These findings are confirmed with inclusion of the PREDNOS study (**Supp. Fig. 1**) [45]. While *post-hoc* analyses in two studies suggest a trend for benefit with prolonged therapy in young children, this finding requires confirmation [45,46].

Based on pharmacokinetics and variations by age, prednisolone is preferably dosed by body surface area in children [47]. However, estimation of body surface area involves complex formulae with variable results [48]. Calculation using body weight is convenient, but results in relative underdosing, particularly in young children [47,49]. Underdosing, using weight-based calculations, was associated with increased risk of frequent relapses in some [50,51], but not in all studies [52,53]. Experts therefore prefer to administer prednisolone based on body surface area for young children [47].

Daily prednisolone is administered in single or divided-doses, with similar time to remission [54]. There is no evidence to support therapy with preparations other than prednisone or its active metabolite, prednisolone [55]. Use of deflazacort, betamethasone, dexamethasone or methylprednisolone is not advised. Prednisolone is best given following food; therapy with antacids, ranitidine or proton pump inhibitors is not routinely required.

Guideline 4: Therapy of relapses

We recommend that relapses be treated with prednisolone at 60 mg/m^2/day (2 mg/kg/day; maximum 60 mg) in single or divided-doses until remission (protein trace/nil for 3 consecutive days), followed by 40 mg/m^2 (1.5 mg/kg, maximum 40 mg) on alternate days for 4-weeks. (1C)

Rationale

Almost one-half of the relapses are precipitated by minor infections, usually of the upper respiratory tract.

Treatment of infection may rarely induce remission, avoiding the need for corticosteroid therapy. A relapse has conventionally, albeit empirically, been treated as outlined above, but guidelines vary in the duration of therapy. Remission is achieved by 7-10 days, and daily therapy is seldom necessary beyond 2 weeks. In case of persistent proteinuria, daily therapy with prednisolone may be extended, to maximum of 6-weeks. Lack of remission despite treatment with 6-weeks' daily prednisolone indicates late steroid resistance that requires specific evaluation and management [10].

Dose based on body surface area and weight is associated with similar time to remission and frequency of subsequent relapses [52,53]. Retrospective studies and small RCTs suggest that reduced dose or abbreviated duration of therapy with prednisolone is effective in inducing and maintaining remission (**Supp. Table III**). Well-powered studies are required to evaluate the optimal dose and duration of prednisolone for relapses.

Guideline 5: Management of frequent relapses and steroid dependence

Definition

Frequent relapses are defined by the ISKDC as occurrence of two or more relapses in the first 6-months after initial response, or four or more relapses in a year [3]. These patients are at risk of morbidity associated with multiple relapses and corticosteroid toxicity. The term has been used for over 40-yr, with minor modifications. Additionally, we propose that patients with three or more relapses in any 6-months be also classified as frequent relapsers (**Box I)**. Steroid dependence, as previously defined, includes patients with two consecutive relapses, while receiving or within 2-weeks of discontinuing prednisolone [3,6].

The occurrence of two or more relapses in the first 6-months is usually associated with high frequency of relapses in the subsequent 12-24 months [3]. Patients experiencing 4 relapses annually receive ~165-200 mg/kg (4.6-5.6 g/m^2) prednisolone, corresponding to 0.45-0.55 mg/kg (12.5-15.5 mg/m^2) daily. As 12-weeks' prednisolone therapy for the initial episode (~115 mg/kg; ~3.4 g/m^2) might be associated with adverse effects [55,56], the risk of steroid toxicity in patients with 3 relapses in any 6-months or 4 relapses annually is considerable [57].

Two additional situations might suggest the need for steroid-sparing therapy. The first is a patient with significant steroid toxicity (**Box I**) and fewer relapses (3 relapses/year; 2 relapses in 6-months). The second is the occurrence of two relapses in 6-months during long-term therapy with corticosteroids or steroid-sparing agents. In both instances, it is rational to manage the patients as frequent relapsers, even if they do not satisfy standard definitions. While stable remission (sustained remission or infrequent relapses i.e., upto one relapse in 6-months) during therapy with steroid-sparing agents is acceptable, the definition of failure of therapy depends on the medication, interval between relapses and need for concomitant corticosteroids.

5.1 Choice of therapy

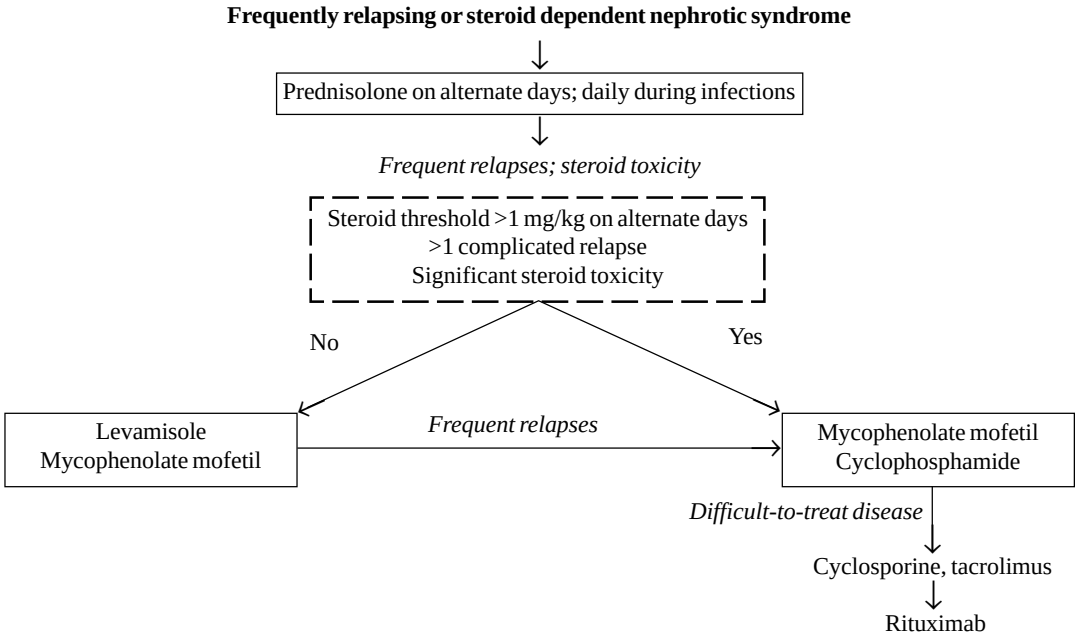
We recommend that the choice of immunosuppressive strategy for patients with frequent relapses be based on considerations of its efficacy and adverse effects, patient age, steroid threshold, severity of relapses and features of steroid toxicity (**Fig. 1**). (X)

Rationale

In patients with frequent relapses, guidelines recommend that corticosteroid therapy for the relapse be prolonged and tapered over 3 months or longer [9,30,58]. The dose at which relapses occur (steroid threshold) is a marker of disease severity. Prolonged therapy with alternate-day prednisolone might maintain remission in patients with low threshold relapses (<0.7 mg/kg on alternate days).

Steroid-sparing interventions are necessary in patients who continue to relapse frequently or show evidence of steroid toxicity while on alternate-day prednisolone (**Fig. 1**). There is limited data on relative efficacy of various steroid-sparing agents, and the choice of immunosuppressive strategy is guided by its efficacy, safety, cost and availability, patient age, disease severity, and parental preference (**Table II**). Potent medications are preferred in patients with high threshold (>1 mg/kg on alternate day) relapses, relapses associated with life-threatening complications, or with significant steroid toxicity (**Box I** and **Table II**). The presence of stable remission (up to one relapse in 6 months) during such therapy is acceptable, and except in severe steroid dependence, prednisolone is tapered and discontinued over few months. Therapy may be modified in patients with frequent relapses or significant adverse effects.

A proportion of patients with SSNS show disease characterized by multiple relapses despite therapy with steroid-sparing agents, and/or medication-associated toxicity. We propose defining difficult-to-treat nephrotic syndrome as patients with: *(i)* frequent relapses or infrequent relapses with significant steroid toxicity; and *(ii)* failure of 2 or more steroid sparing agents: levamisole, cyclophosphamide or MMF. These patients might merit therapy with agents such as CNI and rituximab.



The initial strategy is to administer prednisolone at a dose of 0.5-0.7 mg/kg on alternate days. In patients with stable remission (sustained remission or infrequent relapses), therapy may be tapered to 0.2-0.3 mg/kg on alternate days for 6-12 months. Daily therapy at the same dose for 5-7 days, during minor infections, prevents infection-associated relapses. Patients who relapse at steroid threshold >0.7 mg/kg or show steroid toxicity require therapy with steroid-sparing medications (Table II). The choice of agents is based on disease severity, adverse effects, patient age, cost of therapy, and parental preference. Levamisole or mycophenolate mofetil (MMF) are preferred medications for mild disease. Patients with high steroid threshold (>1 mg/kg on alternate days), complicated relapses and those with significant steroid toxicity (Box I) may be treated with MMF at higher doses (1000-1200 mg/m^2/day) or cyclophosphamide. The use of cyclophosphamide is avoided in children <5-7 yr-old and in peri-pubertal boys due to reduced efficacy and risk of gonadal toxicity, respectively. Patients who relapse despite therapy with two or more steroid-sparing agents (difficult-to treat steroid sensitive disease) are considered for therapy with calcineurin inhibitors, and failing that, rituximab. The use of rituximab is avoided in young children due to the risk of hypogammaglobulinemia.

Fig. 1. **Management of frequently relapsing or steroid dependent nephrotic syndrome.**

While the approach to management indicated in **Fig. 1** suffices in most instances, individual situations may require different preference. Patients diagnosed either with steroid dependence soon after initial therapy, or with significant steroid toxicity at diagnosis of frequent relapses may be considered directly for steroid sparing therapies. Therapy with oral cyclophosphamide is avoided in young patients and in pubertal or post-pubertal boys. Therapy with CNI may be preferred to MMF in very young patients with significant steroid toxicity, even though the definition of difficult-to-treat SSNS is not met.

5.2 Long-term corticosteroids

- In patients with frequent relapses, we suggest tapering prednisolone to a dose of 0.5-0.7 mg/kg on alternate days, for 6-12 months. (2B)
- In patients receiving long term alternate-day prednisolone, we recommend administering the same dose daily for 5-7 days during fever or respiratory tract infection. (1B)

Rationale

Therapy with alternate-day prednisolone is the initial strategy for managing patients with frequent relapses [6,58]. Alternate-day prednisolone, often used as the control limb in RCTs, showed satisfactory response in 43-82.5% patients (**Supp. Table IV**). A balance of benefit over harm is lacking, and there are risks of corticosteroid toxicity. Therefore, in patients in remission at prednisolone dose of 0.5-0.7 mg/kg for a few months, the medication may be tapered to ~0.2-0.3 mg/kg on alternate days. The duration of therapy is at physician discretion, based on its efficacy and assessment of toxicity through monitoring of weight, height, blood pressure, ocular toxicity and hyperglycemia (**Table II**).

Daily prednisolone during infections

More than one-half of relapses in SSNS occur following upper respiratory tract infections. Evidence from three studies (**Supp. Table V)** indicates that, beginning with the onset of infection, switching therapy from alternate-day

to daily administration of prednisolone for 5-7 days prevents the occurrence of relapses. One cross-over trial also supports the use of low-dose daily prednisolone in preventing infection-associated relapses in patients off corticosteroids [59]. Results of the PREDNOS2 trial will clarify the role of these strategies in preventing infection-associated relapses (ISRCTN10900733).

Daily prednisolone in low-dose

Data from an open-label RCT [60] and a case series [61] suggests that low-dose (0.2-0.3 mg/kg) daily prednisolone is associated with fewer relapses than twice the dose (0.5-0.7 mg/kg**)** on alternate days. The strategy led to lower steroid requirement and was not associated with toxicity [60]. These findings require confirmation in studies with longer follow-up that are powered to examine adverse effects, including suppression of the hypothalamo-pituitary-adrenal axis [62].

5.3 Non-corticosteroid therapies

- We recommend use of a steroid-sparing agent in patients failing therapy with alternate-day prednisolone, steroid toxicity or complicated relapses (**Fig. 1**). (1B)
- In patients failing alternate-day prednisolone, we recommend therapy with either levamisole or MMF for 12-24 months. (1B)
- We recommend MMF or cyclophosphamide in patients with significant steroid toxicity, high steroid threshold, complicated relapses, of failure of therapy with levamisole. (1C)

Table II Immunosuppressive Medications for Frequent Relapses

Medication	*Dose*	*Duration*	*Adverse effects*	*Recommended monitoring*
Prednisolone	0.5-0.7 mg/kg on alternate days[a,b]	6-12 mo	Cushingoid features; short stature; hypertension; raised intraocular pressure; glucose intolerance; cataract; elevated transaminases	Screen for side effects, Anthropometry q 3-6 mo; eye evaluation q 6-12 mo; blood sugar and transaminases q 3-6 mo
Levamisole	2-2.5 mg/kg on alternate days	2-3 years	Leukopenia, ANCA positive vasculitis, high transaminases, seizures	Blood counts[c] q 2-3 mo; transaminases q 4-6 mo
Cyclophosphamide	2-2.5 mg/kg/day orally	8-12 weeks	Leukopenia, alopecia, infections; discolored nails; hemorrhagic cystitis; gonadal toxicity and malignancies	Blood counts q 2 weeks[c] Maintain hydration; discontinue during significant infections Co-administer with prednisolone 1 mg/kg AD
Mycophenolate mofetil	600-1200 mg/m^2/d in divided doses; AUC >45 mg.h/L	2-3 years	Abdominal pain, diarrhea, nausea, weight loss; viral warts; leukopenia; elevated transaminases	Screen for adverse effects Blood counts[c] and transaminases q 3-6 mo
Cyclosporine	4-5 mg/kg/day in divided doses; trough 80-120 ng/mL[a]	2-3 years	Both: Nephrotoxicity, hyperkalemia, hepatotoxicity Cyclosporine: Gingival hyperplasia, hypertrichosis; hypertension;	Screen for cosmetic side effects, tremors, diarrhea, hypertension Creatinine, potassium
Tacrolimus	0.1-0.2 mg/kg/d in divided doses; trough 4-8 ng/mL[a]	2-3 years	dyslipidemia Tacrolimus: Tremors, seizures, headache; diarrhea; glucose intolerance; hypomagnesemia	at 2-4 weeks, q 3-6 mo Liver function tests, glucose, uric acid, magnesium and lipids q 3-6 mo
Rituximab	375 mg/m^2, slow IV infusion	2 doses, 1-week apart[d]	Chills, fever; serum sickness; bronchospasm Acute lung injury Neutropenia; *P. jirovecii* pneumonia; reactivation of hepatitis B or JC virus; hypogammaglobulinemia	Pre dose: Blood counts, transaminases; hepatitis and HIV serology; immunoglobulin G (IgG) level Post therapy: CD19 counts; blood counts and IgG

AUC area under the curve (therapeutic drug monitoring); mo months; [a]May reduce dose further if remission is sustained; [b]During infections, administer alternate day prednisolone at 0.5 mg/kg every day for 5-7 d to prevent relapse; [c]Withhold if total leukocyte count <4000/mm^3; [d]One to two additional doses are given at weekly intervals if CD19+ cells are >5/μL (or >1% of CD45+ cells) despite two doses of rituximab.

Rationale

Levamisole: Levamisole has been used for almost 4-decades, mainly in Asia and Europe, as a steroid-sparing agent for frequent relapsing nephrotic syndrome [63]. A meta-analysis (8 studies, 462 patients; **Supp. Table VI**), suggests 35% reduction in the risk of relapses following 6-12 months' therapy with levamisole (RR 0.65; 95% CI 0.48-0.88) [64]. The medication is more useful in patients with frequent relapses than in steroid dependence [65]. Comparative studies indicate that the risk of relapse in patients receiving levamisole is similar to cyclophosphamide (2 studies, 97 children; RR 2.14; 95% CI 0.22-20.95), or MMF (one study, 149 patients; RR 1.11; 95% CI 0.86-1.43) [64]. Given the efficacy and safety, the agent is being examined in two RCTs when administered at onset of the disease (LEARNS, EudraCT 2017-001025-41; NEPHROVIR3, NCT02818738).

Levamisole is given at the dose of 2-2.5 mg/kg on alternate days (**Table II**). While few retrospective studies report its efficacy when administered daily (**Supp. Table VII**), the safety of this strategy should be examined in controlled studies with close monitoring for adverse effects, including neutropenia, raised transaminases, anti-neutrophil cytoplasmic antibodies and/or small vessel vasculitis [63,66,67].

Mycophenolate mofetil (MMF): The use of MMF in frequently relapsing nephrotic syndrome is recent [68]. A review of 7 prospective and 6 retrospective series (508 patients) showed that therapy with MMF for 6-19 months lowered relapse rates, and reduced requirement of prednisolone and/or CNI (**Supp. Table VIII**) [68]. While placebo-controlled, blinded RCTs are lacking, MMF was found to be comparable to levamisole but inferior to cyclosporine in maintaining satisfactory remission or reducing the frequency of relapses in 3 open-label RCTs (**Supp. Table IX**) [64]. Likewise, MMF had efficacy similar or inferior to tacrolimus in a non-randomized comparison (**Supp. Table IX**). MMF is perhaps more efficacious in young children [69], and more effective than levamisole in patients with steroid dependence [70].

Therapy with MMF is given in two divided doses, 600 to 1200 mg/m^2 (20-30 mg/kg) daily [68]. Dose-related adverse effects include leukopenia, abdominal pain and diarrhea. Data from one RCT suggests that patients with higher blood levels of MMF (determined by area under the curve, AUC) show efficacy similar to cyclosporine [71]. Others emphasize the need to achieve mycophenolic acid AUC levels exceeding 45-60 µg*h/mL [72-74] or trough levels >2-3 µg/mL [75-78]. While pharmacokinetics of MMF is variable, adequate levels are achieved with high doses [76-78]. In the absence of facilities for therapeutic drug monitoring, we propose initiating therapy at the lower end of dose range and escalating as tolerated, to 1000-1200 mg/m^2, if the patient continues to relapse.

Cyclophosphamide: Oral cyclophosphamide, at 2-2.5 mg/kg daily for 8-12 weeks, is the most commonly used steroid-sparing agent in SSNS. Its use finds basis in evidence of efficacy and overall safety, as summarized in a systematic review (38 prospective and retrospective studies, 1504 patients) of patients administered cyclophosphamide or chlorambucil [79]. A recent meta-analysis shows reduced risk of relapse at 6-12 months (6 studies, 202 patients; RR 0.44; 95% CI 0.32-0.60) and 12-24 months (4 studies, 59 patients; RR 0.20; 95% CI 0.09-0.46) following therapy with alkylating agents [64]. In comparative studies, the risk of relapse at 12-24 months following cyclophosphamide therapy was similar to levamisole (1 study, 40 patients; RR 1.12; 95% CI 0.86-1.16), but lower than cyclosporine (2 studies, 95 patients; RR 0.51; 95% CI 0.35-0.74) [64]. A Bayesian network analysis (7 reports, 391 patients) showed lowest relapse rates with cyclophosphamide, compared to other medications [80]. Cyclophosphamide is more effective in patients with frequent relapses than in steroid dependence, and in patients older than 5-7 years (**Supp. Table X**).

Therapy with cyclophosphamide is initiated during remission. Prednisolone is given at a dose of ~1 mg/kg on alternate days during therapy with cyclophosphamide; the medication may subsequently be stopped after 1-2 months. Leukopenia is the chief adverse effect, reported in one-third of patients; other concerns are alopecia and the risk of infections (**Table II**). Leukocyte count is monitored every 2 weeks, and therapy withheld if the count falls below 4000/mm^3. Increased fluid intake and frequent voiding prevents hemorrhagic cystitis which, along with nausea and vomiting, is common with intravenous (IV) dosing. The risk of gonadal toxicity is proportionate to the cumulative dose, and appears to be high in pubertal and post-pubertal boys (Tanner stage 2 or more), and lower in girls [30,79,81]. Therapy with chlorambucil is associated with risk of seizures, and is not recommended.

Given concerns of gonadal toxicity and malignancy, therapy with cyclophosphamide is usually administered after failure of levamisole or MMF, and is limited to one 12-weeks' course (cumulative ~168 mg/kg). Occasionally, cyclophosphamide may be the preferred initial steroid-sparing therapy in patients older than 7-yr, particularly in presence of significant steroid toxicity and/or complicated relapses. Limited evidence indicates that cyclophosphamide (500 mg/m^2 monthly IV pulse;

6-doses) is as effective as 12-weeks' oral therapy [64], and may be considered in patients with likely non-compliance to oral therapy.

5.4 Difficult-to-treat steroid sensitive nephrotic syndrome

- We recommend therapy with CNI, either cyclosporine or tacrolimus, in patients with difficult-to-treat SSNS. (1B)
- We recommend therapy with rituximab in patients who have either failed CNI or have received these agents for a prolonged duration. (1C)
- We suggest that therapy with rituximab be administered during disease remission after ruling out acute and chronic infections, and should target B cell depletion. (2B)

Rationale

Calcineurin inhibitors: Observational studies indicate that CNI (cyclosporine 4-6 mg/kg/day, tacrolimus 0.1-0.2 mg/kg/day, in two divided doses) maintain remission and enable steroid-sparing in 60–90% patients with frequent relapses or steroid dependence who have failed treatment with alkylating agents [82-84]. These agents have not been compared to placebo or to each other in controlled studies for SSNS. While one RCT each found that cyclosporine was associated with reduced risk of relapse as compared to prednisolone (104 children; RR 0.33; 95% CI 0.13-0.83) or MMF (see above), patients relapsed when the therapy was discontinued [64]. In view of the efficacy and significant steroid-sparing, CNI are preferred for patients with high threshold relapses or significant corticosteroid toxicity. While therapy with CNI is usually restricted to patients with difficult-to-treat SSNS (**Box I**), these agents may be considered before MMF or cyclophosphamide in young children with severe steroid dependence and/or significant steroid toxicity. The choice of the medication should follow discussion with parents about potential toxicities and the need for monitoring.

Chief adverse effects of CNI include acute and chronic nephrotoxicity (with both agents), hirsutism, gum hypertrophy, hypertension and hyperlipidemia (with cyclosporine), and hyperglycemia or seizures (with tacrolimus) [82,83]. While tacrolimus is preferred to cyclosporine due to lack of cosmetic effects, only the latter is available as an oral suspension for young children. Therapy should be administered for at least 12-months, with monitoring of drug levels (**Table II**). Lower target trough levels and once-daily dosing is acceptable during sustained remission [85.86]. The role of protocol biopsies, before initiating therapy with CNI and following their prolonged use, is discussed in Guideline 2.

Rituximab: B cell depletion has emerged as an effective strategy for sustaining remission in patients with steroid- and/or CNI-dependent nephrotic syndrome. Therapy with rituximab (375 mg/m^2 IV once a week for 1-4 doses) in 13 prospective and retrospective series (n=159) led to sustained remission in 25-71% patients, postponement of relapse by (median) 5-11 months, and withdrawal of other therapies [87]. A systematic review confirmed similar efficacy in 86 adults administered rituximab for frequent relapses [88]. In non-randomized comparisons, the efficacy of rituximab was superior to cyclophosphamide (2 studies, 148 patients) and comparable to tacrolimus (1 study, 23 patients) (**Supp. Table XI**). In a prospective study, therapy with 2-3 doses of rituximab in 101 patients was associated with over two-third reduction in relapses, postponement of relapse by median 16-months and reduced steroid requirement [89].

Data from 7 RCTs in patients with frequent relapses and steroid/CNI dependence indicates superior efficacy of rituximab as compared to placebo (2 studies, 71 patients), or no additional therapy (2 studies, 91 patients); the efficacy was similar or superior to CNI in one study each (174 patients) (**Supp. Table XI**). A Cochrane meta-analysis concluded that therapy with rituximab, in combination with CNI and prednisolone, versus the latter alone, reduced the risk of relapse at 6 months (5 studies, 269 patients; RR 0.23, 95% CI 0.12-0.43) and 12 months (3 studies, 198 patients; RR 0.63, 95% CI 0.42-0.93) [64].

Experts advise administering rituximab at a dose of 375 mg/m^2 IV, using B cell depletion (CD19+ cells <1% of CD45+ cells, or <5 cells/μL) as a marker for adequacy of dosing. While B cell depletion is usual after even one dose [87], a maximum of 4 infusions have been given. Since administration of rituximab during relapse is associated with its urinary excretion and reduced half-life, therapy is preferred during remission [90]. B cell recovery usually occurs by 6-9 months, and is associated with risk of relapses [87,88,90]. Studies comparing response to rituximab in relation to the number of doses and use of maintenance immunosuppression are summarized in **Supp. Table XII**. An international cohort on 511 patients with frequent relapses or steroid dependence showed that relapse-free survival was significantly shorter for patients given a single dose of rituximab (8.5 months) compared to those given two (12.7 months) or more doses (14.3 months) [91]. Additional immunosuppression was useful in sustaining remission following therapy with a single dose of rituximab. In patients with difficult-to-treat SSNS with satisfactory response to rituximab, repeated doses of the medication, following relapses or repopulation of B cells, is suggested as a strategy to sustain remission

(**Supp. Table XII**). Given the concerns discussed below, the optimal strategy is still not clear.

Systematic reviews show that therapy with rituximab is associated with infusion reactions (4 studies, 252 children; RR 5.8, 95% CI 1.3-25.3) [64], delayed adverse events and infections [87,88]. A German registry of autoimmune diseases (370 patients) reported serious infections in 5.3 cases per 100 patient-years [92]. Patients with lymphoma treated with rituximab show reactivation of hepatitis B virus infection in 9% (95% CI 5%-15%) patients [93]. In contrast to the reports of normal IgG in adult patients receiving multiple doses of rituximab (**Supp. Table XII**), hypogammaglobulinemia is not uncommon in children with nephrotic syndrome and autoimmune diseases. The risk of hypogammaglobulinemia correlates inversely with age, and positively with the number of rituximab doses [94-96].

We recommend that rituximab be used in patients with difficult-to-treat disease, under the supervision of a pediatric nephrologist. Its use should be avoided in young children (<5-7 yr old), and restricted to patients failing other steroid-sparing agents. Active acute infections and chronic viral infections should be ruled out before therapy. We recommend administering two doses of rituximab during disease remission, at 375 mg/m^2 one-week apart, followed by confirmation of B cell depletion, 2-7 days after the second dose. Vigilance for infections and monitoring for leukopenia and hypogammaglobulinemia is essential during follow up. Further doses of rituximab should be avoided in patients with severe infusion-related adverse events, severe infections or with hypogammaglobulinemia. Prophylactic antibiotics are not routinely recommended. We suggest administering cotrimoxazole (150 mg/m^2 or 5 mg/kg of trimethoprim on alternate days) in patients receiving additional immunosuppression, such as those receiving maintenance treatment with CNI or MMF following therapy with rituximab.

SUPPORTIVE CARE

Patients with nephrotic syndrome are at risk of complications of the disease, and side effects of its medications. Principles of management of hypertension, thromboembolism, growth retardation, obesity, dyslipidemia, and hypothyroidism are discussed in the guidelines on steroid resistant nephrotic syndrome [10]. We emphasize that patients who have received oral steroids for more than 2-weeks within the past one-year, should receive additional corticosteroids during conditions associated with physiological stress like systemic infections, inadequate oral intake, lethargy, dehydration, invasive or dental surgery, trauma and large burns [10]. Conditions such as uncomplicated viral infections, acute otitis media and fever following immunization do not require stress dosing with steroids.

Guideline 6: Management of Hypovolemia and Edema

Edema, a cardinal feature of nephrotic syndrome, often requires specific therapy. We propose that edema be empirically classified based on appearance and percentage weight gain from baseline, as mild (≤7% increase), moderate (8-15%) and severe (>15% increase) [97]. If urine protein is monitored regularly, the occurrence of more than mild edema is unusual. Patients with severe edema have marked hypoalbuminemia (serum albumin <1.5 g/dL), along with ascites and anasarca that interferes with daily activities [97,98]. Intravascular volume depletion is common in patients with moderate or severe edema [99,100], and should be assessed before instituting therapy with diuretics.

6.1 Hypovolemia

- We recommend that patients with moderate to severe edema be assessed for intravascular volume status before initiating therapy with diuretics (**Fig. 2**). (X)
- We recommend the use of normal saline and IV albumin in patients with disease relapse and hypovolemia. (1C)

Rationale

A combination of clinical and biochemical features helps estimate intravascular volume (**Box III**, **Fig. 2**) [97,101]. Patients with hypovolemia often have abdominal pain, nausea, vomiting, dizziness and lethargy. Examination shows tachycardia, pallor, cold peripheries, delayed

Box III Features of Hypovolemia During Relapse

Clinical features

Abdominal pain, vomiting, lethargy

Prolonged capillary refill time; cold extremities

Tachycardia, low volume pulses

Low blood pressure; postural hypotension

Biochemical indices

Elevated hematocrit

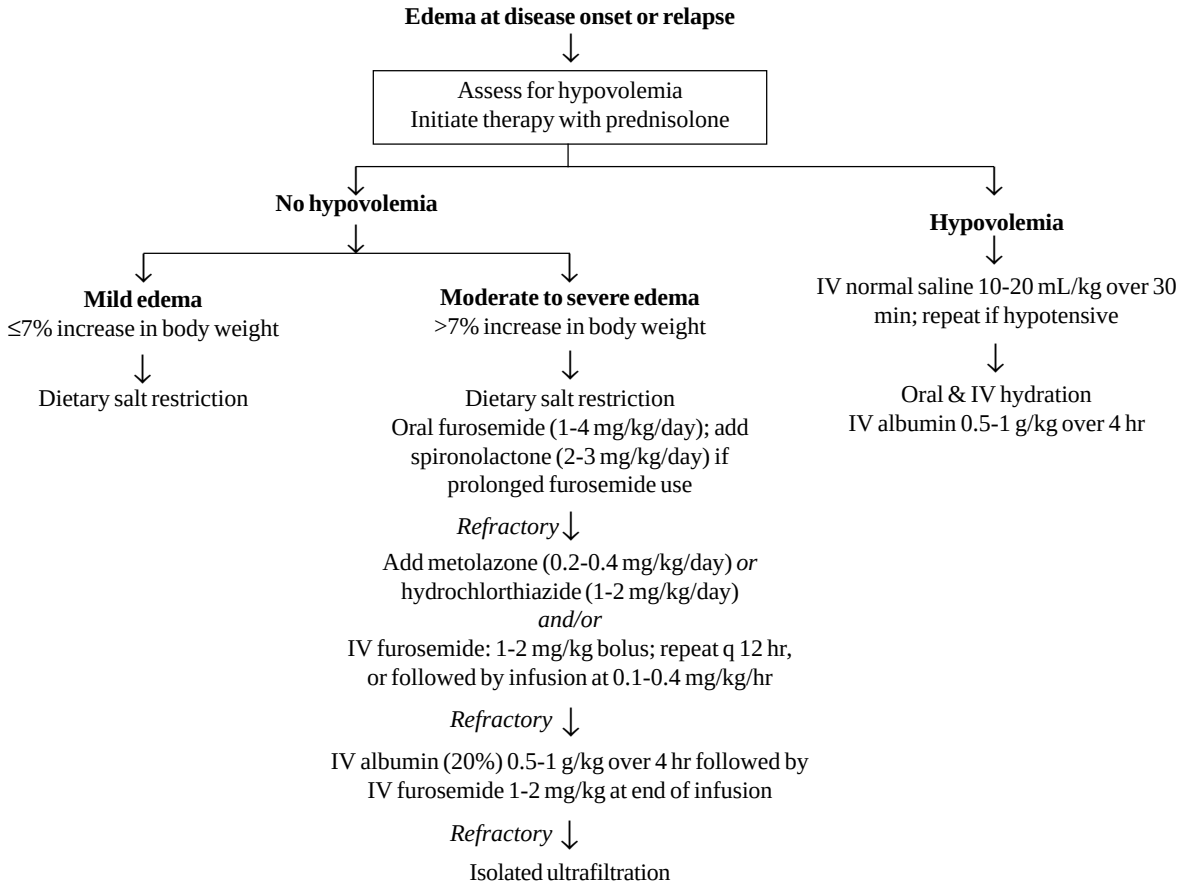
Blood urea (mg/dL) to creatinine (mg/dL) ratio >100

Fractional excretion of sodium <0.5%

Urinary potassium index [urine K^+/(urine Na^++K^+) >0.6]

Ultrasonography: decreased inferior vena cava diameter, increased collapsibility index [110]

$$\textit{Fractional excretion of sodium} = \frac{\textit{urine } Na^+ \times \textit{serum creatinine} \times 100}{\textit{serum } Na^+ \times \textit{urine creatinine}}$$



Edema is empirically defined, based on increase in body weight, as mild, moderate and severe (>15% increase). Patients with mild edema are managed with salt restriction alone; prednisolone therapy is associated with spontaneous diuresis within a few days. Hypovolemia should be excluded (Box III) before considering therapy with diuretics. Oral furosemide is the diuretic of choice; patients receiving therapy with furosemide for >48-hr should additionally receive a potassium-sparing diuretic. Edema refractory to furosemide therapy may be treated with additional thiazide diuretics or IV furosemide, as bolus and/or infusion. Combination therapy with IV albumin (20%) and furosemide enables diuresis in patients refractory to the above measures. IV albumin carries the risk of fluid overload and pulmonary edema in patients with renal dysfunction. Patients with features of hypovolemia require bolus(es) of normal saline if hypotensive, followed by oral and IV hydration, and IV albumin (20%) infused over 2-4 hr.

Fig. 2. Management of edema in nephrotic syndrome.

capillary refill and postural hypotension, and rarely shock [97,101,102]. On the other hand, patients with hypervolemia have refractory anasarca, hypertension and dyspnea [99,100]. Two urinary indices may help assess intravascular volume: fractional excretion of sodium (FENa) and potassium index [103,104]. While both underfill and overfill states are associated with sodium retention [105-107], FENa <0.5% and potassium index >0.6 indicate high aldosterone activity, characteristic of hypovolemia [104,105,108]. The indices are not reliable with recent diuretic therapy and while receiving IV fluids. Other parameters of volume status include changes in hematocrit, urea to creatinine ratio [105], inferior vena cava diameter and collapsibility, and bioimpedance analysis [97,99-101,109,110].

Hypovolemia may occur at disease onset or relapse, particularly in a setting of diarrhea, vomiting or unsupervised diuretic therapy. Therapy with diuretics should be discontinued. Hypotensive patients should receive 1-2 boluses of isotonic saline (10-20 ml/kg infused over 20-30 minutes) and/or 5% albumin (10–15 ml/kg over 30-60 minutes) (**Fig. 2**). Subsequently, patients are managed with IV and oral hydration, and IV albumin (20%; 0.5–1 g/kg over 3-4 hr) [97,99,101].

6.2 Edema

- We recommend oral furosemide as first line therapy for patients with moderate edema without hypovolemia (**Fig. 2**). (1C)
- We suggest that patients with furosemide-refractory

edema be managed as follows: *(i)* combination of loop diuretics with thiazide; *(ii)* co-administration of human albumin with IV furosemide. (X)

Rationale

Patients with mild edema do not require diuretic therapy. Corticosteroid therapy for relapse results in diuresis within one-week, enabling loss of retained extracellular fluid [97,101]. Patients are advised to limit sodium intake (1-2 mEq/kg/day; 15-35 mg/kg salt). Foods rich in salt (>10 mEq/100 g; e.g., bread, cornflakes, processed cheese, sauces, potato chips, salted nuts, *papad*, pickles) and preserved foods (canned vegetables, soups and meat) are avoided in presence of significant edema [97,101].

Diuretics are the initial therapy for patients who are volume replete. Patients with moderate edema without hypovolemia are managed with furosemide (2-4 mg/kg/day) that acts on the ascending limb of Henle [101,105]. Sequential nephron blockade, with additional use of hydrochlorothiazide (2-4 mg/kg/day) or metolazone (0.1-0.2 mg/kg q12-24 hr), augments diuresis by reducing distal sodium reabsorption [97,101]. Monitoring for hypovolemia, hypokalemia and alkalosis is essential. Spironolactone has limited diuretic efficacy, but is an effective potassium-sparing agent in patients receiving high-dose furosemide [97,101]. Use of acetazolamide or amiloride is not advised.

Patients with severe edema may fail to respond to maximal doses of furosemide and thiazide diuretics (diuretic resistance) [98]. Factors contributing to diuretic resistance are poor adherence to salt restriction, reduced bioavailability of furosemide, hypoalbuminemia, hypovolemia, and compensatory salt reabsorption in the distal tubule. The bioavailability of oral furosemide is 20-60%, and is impaired by gut edema in nephrotic syndrome [98]. In patients unresponsive to oral furosemide, assessed as absence of diuresis within 2-4 hr of its administration, switching to IV therapy may elicit a response. IV furosemide, given either as 1-2 mg/kg q 8-12 hr, or bolus of 1 mg/kg followed by infusion of 0.1-0.4 mg/kg/hr is effective [97,98,101]. While torsemide has better efficacy and bioavailability than furosemide in adults with heart failure [111], information in nephrotic syndrome is lacking.

Furosemide, tightly bound to blood albumin, is actively secreted *via* organic acid pumps in the ascending limb of Henle. Tubular secretion is impaired in patients with severe hypoalbuminemia, resulting in diuretic resistance [101]. The combination of 20% albumin (0.5-1 g/kg infused over 3-4 hr) and furosemide (1-2 mg/kg at end of infusion) enhances drug delivery to tubules, with increased efficacy in terms of urine output and weight loss [110,112,113]. A meta-analysis confirmed that combination therapy results in diuresis and natriuresis, which declines by 24-hr [101,114]. Therapy with IV albumin may be associated with risk of worsening hypertension, respiratory distress and heart failure, and is therefore avoided in patients with impaired kidney function [97-99,101,112].

Patients with severe edema who are refractory to the above therapies are likely to have fluid overload, usually in presence of steroid resistance or kidney dysfunction. These patients might require ultrafiltration or kidney replacement therapy. An approach to evaluation and management of edema is shown in **Fig. 2**.

Guideline 7: Infections and Immunization

7.1 Bacterial infections

We suggest that serious bacterial infections associated with nephrotic syndrome be managed as indicated in **Table III**. (X)

Rationale

Infections are the chief complication in patients with SSNS, accounting for 19-44% of hospitalizations [115-120]. Contributing factors include the use of immunosuppressive agents, anasarca, and urinary losses of IgG and complement factors, that predispose to infection with encapsulated organisms [121]. Peritonitis is the most common severe infection, followed by pneumonia and cellulitis [115-119]. Chief pathogens causing peritonitis are pneumococci and *E. coli;* those causing pneumonia include pneumococci, *H. influenzae* and *S. aureus*; and those responsible for cellulitis are staphylococci, group A streptococci and *H. influenzae* [115-119]. The diagnosis and treatment of severe infections should follow standard guidelines [122-124] (**Table III**). Apart from vaccines, there is no evidence of efficacy of other interventions for preventing bacterial infections in patients with nephrotic syndrome [125].

Viral infections

Several viruses, including rhinovirus, adenovirus, influenza, parainfluenza, enterovirus, and respiratory syncytial and Epstein Barr viruses, might trigger disease relapses [126,127]. Infections such as varicella, zoster and influenza might be associated with significant morbidity, and merit specific prevention and management [128-130].

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection: Infection with SARS-CoV2, the etiological agent of coronavirus disease (COVID-19), poses challenges in management of patients with nephrotic syndrome [131]. While children show mild

Table III Management of Serious Infections

Infections	*Organisms*	*Diagnosis*	*Treatment*
Peritonitis	*S. pneumoniae*, *S. pyogenes* *E. coli*, Gram negative bacteria	Ascitic fluid: >100 white cells/mm^3, >50% neutrophils Ascitic fluid: Culture, latex agglutination, PCR	Ceftriaxone or cefotaxime for 7-10 d Ampicillin and gentamicin/amikacin for 7-10 d[a]
Pneumonia	*S. pneumoniae*, *S. aureus*, *H. influenzae*	Chest X ray; blood culture; sputum for Gram stain and culture	Oral: Amoxicillin, coamoxiclav, cefuroxime for 10-14 d[a] Parenteral: Ceftriaxone; or ampicillin and amikacin for 7-10 d[a]
	Influenza H1N1	Throat swab for H1N1 by PCR	Oseltamivir for 5 d
	M. tuberculosis	Tuberculin test; pleural tap, gastric aspirate, sputum: acid fast bacilli, CBNAAT	Therapy as per National Tuberculosis Elimination Program [16]
Cellulitis	*S. aureus*, *S. pyogenes* *H. influenzae* Gram negative bacteria	Pus for culture, sensitivity Blood culture	Parenteral: Coamoxiclav; cloxacillin with ceftriaxone for 7-10 d[a]
Sepsis	*S. pneumoniae*, Gram negative bacteria	Complete blood counts; C-reactive protein, procalcitonin; blood culture	Ceftriaxone and amikacin for 10-14 d[a]
Varicella	Varicella zoster virus	Clinical	IV acyclovir (1500 mg/m^2/day in three doses) or oral acyclovir (80 mg/kg/day in four doses) for 7-10 d

CBNAAT-cartridge based nucleic acid amplification test; PCR-polymerase chain; [a]Penicillin allergy: Clarithromycin, azithromycin, clindamycin or vancomycin.

disease, patients on immunosuppression constitute a high-risk group that is predisposed to adverse outcomes. Affected patients are at risk of AKI, particularly if associated with hypovolemia or aggressive use of diuretics. In absence of specific therapy for SARS-CoV-2 infection, most expert groups advise reduction of immunosuppression to acceptable levels, balancing the risk of disease relapses against infection [131,132]. Other considerations include advice through teleconsultation; low threshold for inpatient monitoring of infected patients; and limiting the use of biological agents and antimetabolites [131,132]. Steroid dosing during SARS-CoV-2 infection should follow standard practices regarding stress dosing [10]; relapses may be treated with a lower dose of prednisolone.

7.2 Immunization

We suggest that patients with nephrotic syndrome receive: *(i)* age-appropriate killed, subunit or inactivated vaccines; *(ii)* live vaccines following principles outlined in **Table IV**; *(iii)* vaccines against pneumococcus, varicella, influenza and hepatitis B (**Table V**). (X)

Rationale

Children with nephrotic syndrome should receive vaccines as appropriate for age [133,134]. Killed, inactivated or subunit vaccines are not contraindicated, but may have reduced efficacy during immunosuppression [133-136]. Principles of immunization with live vaccines in immunocompromised children and their household contacts are listed in **Table IV** [124,134,137]. The schedule for administration of specific vaccines that are relevant to patients with nephrotic syndrome is summarized in **Table V** [133,134,138]. The risk of relapse following vaccination is negligible [135,139].

Pneumococcal vaccine

The availability of safe and immunogenic vaccines has reduced the risk of pneumococcal infections in patients with relapsing nephrotic syndrome [140]. Two categories of vaccines are available. The polysaccharide vaccine (PPSV23) is poorly immunogenic in patients younger than 2-years, and does not induce immunological memory. Conjugate vaccines (PCV7-, 10- and 13-valent) induce superior and sustained antibody responses and immune memory even in young infants, with pooled efficacy of 58% (95% CI 29-75%) against invasive disease caused by any pneumococcal serotype [135,141]. The efficacy of PPSV23 and PCV vaccines in patients with SSNS is variable. Information is lacking on the precise impact of vaccination on rates of peritonitis, cellulitis and pneumonia.

Table IV Principles of Immunization with Live Vaccines in Patients with Nephrotic Syndrome

Immunosuppression	*Advice*
Receiving high dose prednisolone (≥2 mg/kg/d; ≥20 mg/day if >10 kg) for <14 d	Vaccinate immediately after discontinuing treatment
Receiving high dose prednisolone (≥2 mg/kg/d; ≥20 mg/day if >10 kg) for ≥14 d	Vaccinate 1-month after discontinuing corticosteroids
Receiving low-moderate dose prednisolone (<2 mg/kg/d or equivalent; <20 mg/d)	No live vaccines until discontinuation of steroid therapy
Low-dose alternate day prednisolone and pressing need for vaccine	Live vaccine may be administered
Patients receiving cyclophosphamide	Avoid live vaccines until off therapy for 3 months
Patients receiving calcineurin inhibitors, levamisole or mycophenolate mofetil	Avoid live vaccines until off therapy for 1 month
Therapy with rituximab	Avoid live vaccines until after B-cell recovery (~6-9 months)
Immunocompetent siblings and household contacts	Do not administer oral polio vaccine; may receive measles-mumps-rubella, rotavirus and varicella vaccines
Household contacts older than one year	Administer influenza vaccine annually

Table V Specific Vaccines for Patients with Nephrotic Syndrome[a]

Vaccine	*Age*	*Previously received*	*Vaccine*	*Schedule*
Pneumococcal: Conjugate (PCV, 13-valent preferred to 10-valent)	6-72 mo	Completely immunized (3 doses at 6, 10, 14 wks; booster at 12-15 mo)	PCV13/10 PPSV23	One dose ≥2-yr-old One dose when ≥2-year-old and ≥8 wk after last PCV13/10 dose[b]
Polysaccharide, (23-valent, PPSV23)		No or incompletely immunized	PCV10/13 PPSV23	Two doses, ≥8 weeks apart[c] One dose when ≥2-yr-old and ≥8 wk after last PCV13/10 dose[b]
	>72 mo	Completely immunized No or incompletely immunized	PPSV23 PCV10/13 PPSV23	1 dose[b] 1 dose 1 dose, ≥8 wk after last PCV13/10 dose[b]
Varicella[d]	>15 mo	No evidence of immunity[e]	Live attenuated	Two doses 4-8 wk apart
Influenza	>6 mo		Inactivated	Annually
Hepatitis B	Any	No, or anti-HBs <10 mIU/mL	Subunit (10 µg/0.5 mL)[f]	3 doses at 0, 1 and 6 mo; or in an accelerated schedule with ≥4 wk gap between doses 1 & 2, ≥8 wk between doses 2 & 3, and ≥16 wk between doses 1 & 3[f]

[a]Efficacy of vaccines might be attenuated while on high dose corticosteroids or other immunosuppression; [b]Repeat after 5-yr if still experiencing disease relapses; [c]If the two doses are administered at <1-yr-old, give one additional dose during second year of life; [d]Avoid in patients <15 months; administer while off immunosuppression (Table IV); [e]Immunity refers to past diagnosis of varicella or herpes zoster, verified by a physician; documented receipt of 2-doses of vaccine 4-8 weeks apart; or serological evidence of immunity; [f]Consider post-vaccination testing for adequacy (anti-HBs antibody ≥10 mIU/mL) and administering higher (20 µg) or additional doses

Both PCV7/10/13 and PPSV23 elicit satisfactory serological response, even when given during relapse or while on immunosuppressive agents [135]. Nevertheless, we suggest that the vaccine be preferably given during remission, and while on low or no immunosuppression. Antibody responses are ill-sustained in patients with recurrent relapses, justifying re-dosing with PPVS23 after 5 years if the disease remains active; more than 2-doses of PPSV23 are not recommended [134,135].

Varicella vaccine

In view of the risk of severe disease in immunocompromised patients, we recommend that patients with nephrotic syndrome receive two doses of the varicella vaccine, 4-8 weeks apart (**Table V**) [134,138]. Two doses result in seroconversion in ~95% vaccinees; breakthrough varicella might occur in 2.2-7.3% children [142]. The vaccine was safe and immunogenic in 109 patients with nephrotic syndrome,

including those receiving low-dose corticosteroids, in two prospective series [143,144] and in an open-label RCT [145].

Severe varicella might follow infection in at-risk individuals exposed to persons with either varicella or herpes zoster. Multiple strategies for post-exposure prophylaxis are used to prevent viral transmission (**Table VI**) [124,133,134,138,146-149]. Unimmunized patients with nephrotic syndrome who are not immunosuppressed should receive the vaccine within 5-days of exposure [124]. The risk of post-exposure varicella was reduced to one-third in children who were vaccinated following exposure, compared to those unimmunized (3 studies; *n*=110; 23% *vs.* 78%) [147]. Healthy household contacts should also receive the vaccine to minimize the risk of infecting the patient. In patients in whom vaccination is contraindicated, the Center for Disease Control recommends administration of varicella zoster immune globulin (VARIZIG) within 10-d of exposure [148]. VARIZIG administration was associated with varicella in <10% of 507 high-risk participants, including 231 immunosuppressed children [149]. In view of the low and variable titer of anti-VZV antibodies [150], intravenous immunoglobulin (IVIG) is not recommended [124,134]. If VARIZIG is not available, similar to guidelines from the American Academy of Pediatrics [124] and French Society of Pediatric Nephrology [138], we recommend administering oral acyclovir or valacyclovir for 7-days, starting 6-10 days after exposure, corresponding to the period of secondary viremia (**Table VI**).

Influenza vaccine

Influenza accounts for 13% of all pneumonia, and 7% of severe pneumonia in children <5-yr-old [150,151]. Approximately 1 in 5 unvaccinated children are annually infected by influenza, of which one-half are symptomatic [152]. Given the risk of morbidity in immunosuppressed individuals, annual administration of the inactivated influenza vaccine is recommended for patients with nephrotic syndrome (**Table V**), and their household contacts [124,130,138].

Hepatitis B vaccine

Hepatitis B vaccination coverage rates in India are unsatisfactory, and 45% of 1-6 yr-old children are not vaccinated [153]. Compared to healthy children, fewer patients with nephrotic syndrome show seroprotective (≥10 mIU/mL) antibody titers [154]; one-half of these patients seroconvert after vaccination [136,155]. Seroprotection was lower in patients with steroid resistance, and those on non-steroid therapies [136,154,155]. To overcome vaccine failure, we advise an accelerated schedule using twice the age-appropriate dose, and assessment of serological response to administer booster dose(s) (**Table V**) [156].

GUIDELINE 8: TRANSITION OF CARE

We recommend that patients with nephrotic syndrome who continue to have relapses in adolescence be transitioned into care by adult physicians. (X)

Rationale

SSNS is a self-limiting illness, with the majority of patients outgrowing the illness by puberty. Review of information from multiple cohorts, with median follow-up of 4-30 yr, indicates that the frequency of relapses declines with age [3,4,157-159]. However, 5-42% patients may continue to have active disease in adulthood. Risk factors for illness persisting beyond

Table VI Post-Exposure Management of Unimmunized Patients with Nephrotic Syndrome Exposed to Varicella[a]

Contraindication to live vaccine[b]	*Strategy*	*Timing after exposure*	*Level of evidence*
No	Administer varicella vaccine	As soon as possible, <5 d	A [133,146,147]
Yes	*Options (in order of preference)* Varicella zoster immunoglobulin (VARIZIG),[c] 125 IU per 10 kg body weight (maximum 625 IU) intramuscular	<10 days; preferably <4 days	B [148,149]
	Oral acyclovir, 80 mg/kg in 4 divided doses (maximum 3.2 g) daily for 7 days OR oral valacyclovir (if ≥3-mo-old), 60 mg/kg (maximum 3 g) daily in 3 divided doses for 7 days	Begin 6-10 d after exposure	C [124,134,138]
	Intravenous immune globulin, 400 mg/kg	<10 d	X [124,134]

[a]More than 5 minutes of face-to-face contact with individual with varicella or zoster, while indoors; [b]See Table IV; [c]Available internationally from one manufacturer since 2006 when VZIG was discontinued (https://varizig.com/liquid-ordering_info.html); brands marketed in India include Vartiect-CP from Paviour Pharma)

18-yr of age include early age at onset, and frequently relapsing or steroid dependent course [3,4,157,158].

Major infections, associated with relapses and intense immunosuppression, are the chief cause of hospitalization and mortality (0-8%) [3,157,158]. Kidney failure is uncommon (<1%) in patients with SSNS. There is significant risk of short stature (15%), obesity (10%), hypertension (6-46%), metabolic bone disease (9-63%), diabetes mellitus (2%), ocular complications (10%), infertility and malignancies [157,158,160]. Psychosocial concerns, including school drop-out, unemployment and unstable relationships are common [161].

Given the risk of disease persistence and prevalence of complications, it is advised to transfer the care of adolescents with relapsing disease to 'adult' nephrologists by 18 year of age. National and international guidelines advocate for smooth transition, with emphasis on shared clinics and consideration of patient and parent perspectives [162].

CONCLUSIONS

The present guidelines, based on best available evidence and expert guidance, provide directions for evaluation and management of SSNS in children. Recommendations, proposed by the Indian Society of Pediatric Nephrology, in 2001 and 2008, have been revised based on systematic reviews, published studies and expert opinion. The management of frequent relapses continues to be challenging, with morbidities associated with the disease as well as therapies. Well-designed prospective studies are required to address issues related to therapy of the initial episode and relapsing nephrotic syndrome **(Table VII)**. We hope that the present guidelines will standardize therapies and improve the quality of care for patients with the disease.

Table VII Areas for Clinical Studies in Steroid Sensitive Nephrotic Syndrome

Therapy of initial episode, relapse
Optimal dose and duration of corticosteroid therapy in young (<4-6 years) patients.
Optimal intensity of therapy with prednisolone (daily and alternate day dose and duration) to induce remission and reduce further risk of relapses.
Management of frequent relapses
Efficacy and safety of prednisolone administered on alternate days or daily; minimum effective dose.
Relative efficacy and safety of various immunosuppressive agents.
Efficacy and long-term safety of therapy with calcineurin inhibitors; lowest effective dose.
Efficacy and long-term safety of therapy with rituximab; optimal dosing strategy (redosing at relapses, sequential administration vs maintenance immunosuppression); safe cumulative dose threshold.

Note: Supplementary material related to these recommedations is available with the online version at *www.indianpediatrics.net*

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ANNEXURE I

Expert Group of Indian Society of Pediatric Nephrology

Participants: Anil Vasudevan, *Bengaluru;* Abhijeet Saha, *New Delhi*; Aditi Sinha, *New Delhi*; Aditi Joshi, *New Delhi*; Aliza Mittal, *Jodhpur;* Amarjeet Mehta, *Jaipur;* Arpana Iyengar, *Bengaluru;* Arpita Gogoi, *Dibrugarh;* Anand S Vasudev, *New Delhi;* Pankaj Hari, *New Delhi;* Ranjeet Thergaonkar, *Mumbai;* Priyanka Khandelwal, *New Delhi;* Girish C Bhatt, *Bhopal;* Indira Agarwal, *Vellore;* Jitendra K Meena, *New Delhi;* Jyoti Sharma, *Pune;* Kanika Kapoor, *New Delhi;* Kamran Afzal, *Aligarh;* Kanav Anand, *New Delhi;* Karalanglin Tiewsoh, *Chandigarh;* Kirtisudha Mishra, *New Delhi;* M Ashraf, *Srinagar;* Manish Kumar, *New Delhi;* Manisha Sahay, *Hyderabad;* Mukta Mantan, *New Delhi;* OP Mishra, *Varanasi;* PK Pruthi, *New Delhi;* Rajiv Sinha, *Kolkata* Shobha Sharma, *New Delhi;* Subal Pradhan, *Cuttack;* Sudha Ekambaram, *Chennai;* Susan Uthup, *Thiruvananthapuram;* Sanjeev Gulati, *New Delhi;* Saroj K Patnaik, *New Delhi;* Sriram Krishnamurthy, *Puducherry;* Suprita Kalra, *New Delhi;* Sushmita Banerjee, *Kolkata;* Vinay Agarwal, *New Delhi;* Sumantra Raut, *Kolkata;* Arvind Bagga, *New Delhi, India.*

Experts: Uma Ali, *Mumbai*; Kumud Mehta, *Mumbai*; Madhuri Kanitkar, *New Delhi*; Amit K Dinda, *New Delhi*; Geetika Singh, *New Delhi*; Kishore D Phadke, *Bengaluru*; BR Nammalwar, *Chennai*; RN Srivastava, *New Delhi*.

Supplementary Table I Grading of Evidence [i]

Grade	*Quality of evidence*
A	Well designed and controlled studies; meta-analysis on applicable population; true effect lies close to the estimate of the effect
B	Studies with minor limitations; consistent findings from multiple observational studies; true effect is likely to be close to estimate of the effect, but there is a possibility that it is substantially different
C	Single, few or multiple studies with inconsistent findings or major limitations; confidence in the effect estimate is limited, the true effect may be substantially different from estimate of the effect
D	Expert opinion, case reports; very little confidence in effect estimate, true effect likely to be substantially different from the estimate of effect
X	Situations where validating studies cannot be performed, and benefit or harm clearly predominates
Level	*Strength of recommendation*
1	"We recommend": Most patients should receive the recommended course of action
2	"We suggest": Different choices will be appropriate for different patients

Supplementary Table II Recent Randomized Controlled Trials, with Low Risk of Bias, for Initial Episode of Nephrotic Syndrome

Author, yr	*Type, N*	*Predniso(lo)ne (Intervention)*	*Predniso(lo)ne (Control)*	*Follow up, yr*	*Outcomes at 1-2 yr*			
					% relapsing; time to relapse; HR (95% CI)	*% frequent relapsers; HR (95% CI)*	*Relapse rate; RRR (95% CI)*	*Cumulative prednisone, g/m²/yr; MD (95% CI)*
Teeninga 2013 [ii]	Placebo controlled, randomized N=150	60 mg/m² D till remission; 50 mg/m² D for 6-wk; 40 and 20 mg/m² AD for 4-wk each; 10 mg/m² AD for 10-wk [3.4 g/m² in 24-wk]	60 mg/m² D for 6-wk; 40 mg/m² AD for 6-wk; placebo for 12-wk [3.4 g/m² in 24-wk]	≥1.5	80% *vs.* 77%; 8 *vs.* 6 months; NA	59% *vs.* 50%; 1.1 (0.7, 1.8)	1.0 *vs.* 0.6 per yr; 1.2 (0.9, 1.7)	Not available
Sinha 2014 [iii]	Placebo controlled, randomized N=181	2 mg/kg D for 6-wk; 1.5 mg/kg AD for 6-wk; 1, 0.75 & 0.5 mg/kg AD each for 4-wk [3.5 g/m² in 24-wk]	2 mg/kg D for 6-wk; 1.5 mg/kg AD for 6-wk; placebo for 12-wk [2.8 g/m² in 12-wk]	1	53% *vs.* 63%; 9 *vs.* 7 months; 0.57 (0.36, 1.07)	38% *vs.* 40% 1.0 (0.6, 1.7)	1.3 *vs.*1.5 per yr; 0.7 (0.5, 1.1)	2.3 *vs.* 1.9; 0.45 (-0.12, 1.02)
Yoshikawa 2014 [iv]	Open label, randomized N=255	60 mg/m² D for 4-wk; then 60, 45, 30, 15, 7.5 mg/m² AD for 4-wk each [3.9 g/m² in 24-wk]	60 mg/m² D for 4-wk; 40 mg/m² AD for 4-wk [2.2 g/m² in 8-wk]	2	~70% *vs.* 63%; 8 months each; 1.03 (0.76, 1.39)	~50% *vs.* 45%; 1.16 (0.86, 1.56)	1.3 per person-yr each; 1.1 (0.8, 1.4)	6.5 *vs.* 4.6 in 2-yr; *P*<0.001
Webb 2019 [v]	Placebo controlled, randomized N=237	60 mg/m² D for 4-wk; 60, 50, 40, 30, 20, 10 mg/m² AD, 2-wk each [3.2 g/m² in 16-wk]	60 mg/m² D for 4-wk; 40 mg/m² AD 4-wk; placebo 8-wk [2.2 g/m² in 8-wk]	2	80% *vs.* 81%; ~4.5 *vs.* 3.5 months; 0.87 (0.65, 1.17)	50% *vs.* 53%; 1.04 (0.81, 1.35)	3.6 *vs.* 4.0 at 2-yr; 1.1 (0.9, 1.4)	5.5 *vs.* 6.7 at 2-yr; 1.2 (-0.1, 2.5; *P*=0.07)
Sinha 2019 [vi]	Open label, randomized N=160; <4 yr	60 mg/m² D for 6-wk; 40 mg/m² AD 6-wk; 30, 20, 10 mg/m² AD, 4-wk each [4.6 g/m²]	60 mg/m² D for 6-wk; 40 mg/m² AD for 6-wk [3.4 g/m² in 12-wk]	2	Proportions with relapse, other outcomes; results awaited CTRI/2015/06/005939; NCT03141970			
Xu 2020	Placebo controlled, randomized N=154; 1-6 yr	Daily for 6-wk; AD for 6-wk; taper for 12-wk	Daily for 6-wk; AD for 6-wk; placebo for 12-wk	2	Proportions with frequent relapses, other outcomes; results awaited NCT04536181			

AD alternate days; CI confidence interval; D daily; HR hazards ratio; MD mean difference; RRR relative relapse rate; wk weeks; ^rates adjusted for stratifying variables, where reported

Supplementary Table III Studies on Predniso(lo)ne Therapy of Infrequent Relapses

Author, yr	*Type*	*N*	*Prednisone (Intervention)*	*Prednisone (Control)*	*Follow up, months*	*Time to remission; MD (95% CI)*	*% Frequent relapses*	*Cumulative prednisone*
Raja, 2017 [vii]	Retrospective	50	1 mg/kg/d until remission (minimum 7 d), tapered <1-mo	NA	6	<7 days in 70%; 7-10 days in 7%	NA; 0.9±0.8 relapses in 6-mo	0.75±0.25 mg/kg
Fujinaga, 2018 [viii]	Retrospective	49	60 mg/m^2 until remission; tapered AD <6-mo	Comparison: ≤1.8, 1.8-2 and >2 mg/kg/d	12	7, 7.5 & 7 days	39%, 43%, & 55%	NA
Kainth, 2020 [ix]	Open label, randomized	114	60 mg/m^2/d until remission; 40 mg/m^2 AD for 2-wk	60 mg/m^2/d until remission; 40 mg/m^2 AD for 2-wk	12	Not available	23% *vs*. 22%; RD -1 (-17, 14); HR 1.0 (0.8, 1.2)	1.2 (0.3-1.8) *vs*. 1.8 (1.2-2.4) g/m^2***
Borovitz, 2019 [x]	Open label, not randomized	30	1.5 mg/kg/d (A); 1 mg/kg/d (B) until remission; taper 8-10 wk	2 mg/kg/d until remission; tapered 10-12 wk (C)	6	10±5 (A) & 9±3 (B) *vs*. 7±1 days (C)*	NA	43±26 (A), 25±7 (B) *vs*. 46±3 mg/kg*
Sheikh, 2019 [xi]	Open label, randomized	60	1 mg/kg/d until remission; 1.5 mg/kg AD for 4-wk	2 mg/kg/d until remission; 1.5 mg/kg AD for 4-wk	12	9±2 *vs*. 9±2 days; 0.4 (0.7, 1.6) days	NA	12.5 (9-18) *vs*. 17 (14-21) mg/kg**
Kansal, 2019 [xii]	Open label, randomized	40	2 mg/kg/d until remission; 1 mg/kg AD for 4-wk	2 mg/kg/d until remission; 1.5 mg/kg AD for 4-wk	3	Not available	Relapse at 3 months: HR 1.1 (0.4, 3.2)	NA
Raman, 2017 [xiii]	Open label, randomized, equivalence	52[#]	60 mg/m^2/d until remission; 40 mg/m^2 AD for 4-wk	2 mg/kg/d until remission; 1.5 mg/kg AD for 4-wk	6	6.5 *vs*. 6 days	Similar relapse rate	Similar cumulative prednisolone
PROPINE, [xiv]	Open label, randomized, superiority	78	60 mg/m^2/d until remission; 40 mg/m^2 AD for 36 days	60 mg/m^2/d until remission; 40 mg/m^2 AD for 72 days	6	5 (4-7) *vs*. 6 (5-8) days	Not reported; any relapse: 42% *vs*. 58%	1.29 (1.16-1.64) *vs*. 1.33 (127-1.51) g/m^2
Schijvens, 2018 [xv]	Placebo controlled, randomized	144	60 mg/m^2/d until remission; 40 mg/m^2 AD for 2-wk; placebo at 40 mg/m^2 AD for 4-wk	60 mg/m^2/d until remission; 40 mg/m^2 AD for 6-wk	24	Time to first relapse & other outcomes awaited [Reducing STEroids in Relapsing Nephrotic syndrome, RESTERN; NTR5670, EudraCT 2016-002430-76]		

AD alternate days; /d per day; HR hazard ratio; MD mean difference; mo months; NA not applicable; RD risk difference; RR risk ratio; wk weeks; yr year
P<0.05, **<0.01 and ***<0.0001*
[#]*Number of infrequent relapsers among 100 patients randomized*

Supplementary Table IV Controlled Trials on Efficacy of Predniso(lo)ne on Alternate Days (AD) for Frequent Relapses

Author, yr (reference)	*Type of study*	*N*	*Prednisone AD*	*Comparator*	*Follow up, yr*	*Outcomes at 12-24 mo*				*Adverse events*
						Relapses, n or rate	*Proportion (%) with relapses*	*% with frequent relapses*	*Cumulative predniso(lo)ne*	
APN, 1981 [xvi]	Open label RCT	64[#1]	35 mg/m^2	Prednisone at 40 mg/m^2 on 3 consecutive days each week	0.5 (1)[^]	0.9±0.3 *vs.* 1.9±0.4 in 6 months*	43% *vs.* 72%*		3.9±0.2 *vs.* 3.8±0.2 g/m^2 in 6 months	Obesity 57% *vs.* 52%; hirsutism 13% vs. 20%; psychosis 0% *vs.* 8%; infections 17% *vs.* 12%; 4 in each group withdrawn for steroid toxicity
Broyer, 1997 [xvii]	Open label RCT	40	15-20 mg/m^2	Deflazocort in equivalent dose AD	1	3±2 *vs.* 1±1**	88% *vs.* 42%**		5.1 *vs* 5.7 g/m^2	Mean change in height -0.4 *vs.* -0.2 SDS, weight 3.9 *vs.* 1.7 kg & BMD -12 *vs.* -6%; Cushingoid 7 *vs.* 11
Mattoo, 2000 [xviii]	Prospective study	36	0.5-0.8 mg/kg	Prednisolone at same dose; given daily for 5 days during URTI	2	5.5±1.3 *vs.* 2.2±0.9*	Non-relapsers excluded	Not reported	Not reported	Not reported
Jayantha, 2002 [xix]	Open label RCT	129[#2@]	60 mg/m^2 AD, tapered q 4 wk by 10 mg/m^2 (total 7 months)	Prednisolone 40 mg/m^2 AD for 4 wk (total 2 months)	0.5	0.4±0.5 *vs.* 2.1±1.5*	38% *vs.* 88%*	17.5% *vs.* 40.6%*	3.3±1.2 *vs.* 2.7±1.3	Hypertension 30% *vs.* 12.5%; slow growth 35% *vs.* 28.1%
Al Saran, 2006 [xx]	Open label, not randomized	56	<0.5 mg/kg	Levamisole 2.5 mg/kg AD	1	2.6±1.8 *vs.* 1.0±1.8*	100% *vs.* 37.5%*	50% *vs.* 9.4%*	3.9±1.2 *vs.* 3.1±1.9 g/m^2	None *vs.* gastrointestinal symptoms in one patient
Abeyagunawardena, 2008 [xxi]	Placebo-controlled cross-over RCT	40[@]	0.1-0.5 mg/kg; given 5 mg daily for 7 days in URTI	Prednisone at same dose; given placebo daily for 7 days in URTI	2 URTI	Not reported	48% *vs.* 18%*	Not reported	Not reported	No significant events
Gulati, 2011 [xxii]	Open label RCT	100[!]	0.5–0.75 mg/kg	Prednisolone at same dose; daily during infections	1	1.8±0.5 *vs.* 0.9±0.4*	85% *vs.* 61%*	8% *vs.* 4%	138±22 *vs.* 120±32 mg/kg	Not reported
Yadav, 2019 [xxiii]	Open label RCT	61	0.5–0.7 mg/kg	Prednisolone at 0.2-0.3 mg/kg daily	1	1.94 *vs.* 0.55 per person-yr	71% *vs.* 40%	57% *vs.* 7%[$*]	0.39±0.19 *vs.* 0.27±0.07 mg/kg/day	Cataract & glaucoma 6.5% *vs.* 0% each

BMD bone mineral density; NS not significant; RCT randomized controlled trial; SDS standard deviation score; URTI upper respiratory tract infection
[#]Outcomes reported for [1]48 and [2]90 patients; [^]therapy for 6 months; follow up for 6 months more off therapy; [@]included patients with infrequent relapses; [!]includes 32 patients that also received levamisole; [$]includes patients with infrequent relapses with steroid toxicity
*P *<0.05*

Supplementary Table V Studies on Low-dose Predniso(lo)ne Administered Daily at Onset of or During Infections[@]

Author, yr	*Type of study*	*N*[#]	*Intervention: Prednisone*	*Control*	*Duration*	*Outcomes*	
						Relapse rate [RR (95% CI)] or %	*Proportion (%) with relapses*
Mattoo 2000 [xviii]	Non-randomized, prospective study	36	0.5 mg/kg daily x 5 days	Prednisolone 0.5-0.8 mg/kg AD	2 yr	2.2±0.9 *vs.* 5.5±1.3*	Non-relapsers excluded
Abeyagunawardena 2008 [xxi]	Placebo-controlled cross-over RCT	40[$]	5 mg daily x 7 days[@1]	Placebo for 7 days[@1]	2 URTI	Not available	18% *vs.* 48%*
Gulati 2011 [xxii]	Open label RCT	100[^]	0.5-0.8 mg/kg AD; daily x 7 days[@2]	Prednisolone 0.5-0.8 mg/kg AD[@2]	2 yr	0.9±0.4 *vs.* 1.8±0.5 [0.9 (0.4, 1.4)]***	61% *vs.* 85%*
Abeyagunawardena 2017 [xxiv]	Placebo-controlled cross-over RCT	48[#1]	0.5 mg/kg daily x 5 days	Placebo for 5 days	2 yr	Not available	33% *vs.* 58%*
PREDNOS 2 [xxv]	Placebo-controlled RCT	300[#2]	15 mg/m^2 x 6 days (maximum 40 mg)	Placebo for 6 days	Until first infection: 1 yr	Occurrence of relapse [ISRCTN10900733]	

AD on alternate days; CI confidence interval; RR rate ratio; URTI upper respiratory tract infection; yr year
[@]Refers to URTI, except [@1]viral infections and [@2]any infections
[$]While on prednisolone AD
[#]These studies included patients with frequent relapses, except two that also enrolled patients with [1]infrequent relapses and [2]relapsing nephrotic syndrome (≥2 relapses in previous year) while on/off maintenance immunosuppression
[^]Patients requiring prednisolone AD at >1 mg/kg to maintain remission additionally received levamisole at 2-2.5 mg/kg AD
*P *<0.05, **<0.01, and ***<0.0001*

Supplementary Table VI Randomized Controlled Trials Examining Efficacy of Levamisole Administered on Alternate Days

Author, Year	*Type of RCT*	*Comparison**	*N*	*Follow up, months*	*Outcomes at 6-12 months*		
					Proportion (%) with relapse	*Frequency of relapses*	*Relative risk of relapse (95% CI)*
BAPN, 1991 [xxvi]	Placebo controlled	Placebo	61	6	87.1 *vs.* 93.3	Not reported	0.93 (0.79, 1.1)
Weiss, 1993 [xxvii]	Placebo controlled	Placebo	49	12	93.4 *vs.* 88.9	0.7±0.2 *vs.* 0.6±0.3	1.05 (0.86, 1.3)
Abeyagunawardena, 2006 [xxviii]	Open label	No treatment	76	12	19.0 *vs.* 76.5*	Not reported	0.25 (0.13, 0.48)
Gruppen, 2018 [xxix]	Placebo controlled	Placebo	99	12	66.0 *vs.* 85.7*	Not reported	0.77 (0.61, 0.97)
Dayal, 1994 [xxx]	Open label	Prednisone	61	12	40.9 *vs.* 71.4	Not reported	0.57 (0.31, 1.05)
Rashid, 1996 [xxxi]	Open label	Prednisone	40	10	55 *vs.* 90*	Not reported	0.61 (0.4, 0.93)
Sural, 2001 [xxxii]	Open label	Prednisone	58	12	56.7 *vs.* 82.1*	Not reported	0.69 (0.48, 0.99)
Al-Saran, 2006 [xx]	Open label	Prednisone	56	12	41.2 *vs.* 100*	0.1±0.2 *vs.* 0.2±0.2*	0.42 (0.28, 0.63)
Sural, 2001 [xxxii]	Open label	Oral cyclophosphamide	57	12	56.7 *vs.* 37	Not reported	1.53 (0.85, 2.74)
Donia, 2005 [xxxiii]	Open label	Intravenous cyclophosphamide	40	22	64 *vs.* 72	Not reported	0.89 (0.68, 1.16)
Sinha, 2019 [xxxiv]	Open label	Mycophenolate mofetil	149	12	59.2 *vs.* 65.8	1.3 (1.1, 1.7) *vs.* 1.1 (0.3, 1.3)	1.11 (0.86, 1.43)

*P *<0.05*

Supplementary Table VII Non-Randomized Studies Examining Efficacy of Levamisole Administered Daily

Author, Year	*Type of study*	*Dose of levamisole, mg/kg per day*	*Comparison, if any*	*N*	*Follow up, months*	*Outcomes at 6-12 months*			
						Proportion (%) with relapse; frequent relapses	*Frequency of relapses*	*Cumulative prednisone*	*Adverse events (AE)*
Abeyagunawardena, 2017 [xxxv]	Prospective	2.5[#]	AD levamisole (received historically)	58	12	79.3% *vs*. 100%; not reported	2.8±0.8 vs. 1.3±0.9	Median 154.1 *vs*. 254.2 mg/kg	No major AE
Ekambaram, 2014 [xxxvi]	Retrospective	2	Prior year	97	6-24	Effective in 77%	1.3±0.7 vs. 2.4±0.5	2.5±0.69 g/m^2 *vs*. 4.1±0.1 g/m^2	Not reported
Chen, 2010 [xxxvii]	Retrospective	2-3.3	Other agents	12	NA	93.3%; no effect 66.7%	Not reported	Not reported	Not reported
Sumegi, 2004 [xxxviii]	Retrospective	2	Prior year	34	60	32.4% *vs*. 100%; not reported	0.41 vs. 4.4	1.5±1.7 g/yr; 23 off steroids	Neutropenia in 14.7%
Fu, 2004 [xxxix]	Prospective	2-3[#]	AD levamisole, 2-3 mg/kg	36	4-36	17% *vs*. 49%; response in 69% *vs*. 80%	1.3±2.1 vs. 2.0±2.5	0.2±0.4 *vs*. 0.2±0.3 mg/kg/day	Leukopenia in 20% *vs*. 31.3%
La Manna, 1988 [xl]	Prospective	2.5	Levamisole, 2.5 mg/kg, given 2/wk	8	2-16	Response in 25%	Not reported	Not reported	Minimal

NA not available
[#]Having failed AD levamisole

Supplementary Table VIII Non-Randomized Studies on Mycophenolate Mofetil (MMF) in Nephrotic Syndrome

Author, yr (reference)	*Type of study*	*N*	*MMF, mg/m^2 per day*	*Follow up (range), yr*	*Outcomes at 12-24 months*				*Adverse events (AE)*
					Relapses, n or rate	*Proportion with relapses*	*Frequent relapses*	*Predniso(lo)ne, mg/kg per day*	
Bagga, 2003 [xli]	Prospective	19	29 (27.4-30.7)	1	2 (1.2-2.7)	78/9%	15.8%	0.3 (0.2-0.4)	Abdominal pain 26.3%
Gellermann, 2004 [xlii]	Prospective	6	1000	2.1 (1.3-3.3)	Not reported	16.7%	0%	Not reported	Juvenile conglobate acne in 16.7%
Novak, 2005 [xliii]	Retrospective	21	1200	1±0.5	0.47±0.43 per month	80.9%	24%	Not reported	Gastrointestinal AE common but mild; varicella in 4.7%
Al-Akash, 2005 [xliv]	Retrospective	11	948 (500-1087)	1 (0.3-2)	1.05 (0-4.5)	45.5%	18.2%	Not reported	Herpes stomatitis 9.1%; gastrointestinal AE 18.2%
Hogg, 2006 [xlv]	Prospective	33	1200	0.5	1 per 14.7 months	25%	Not reported	Not reported	Leukopenia 15.6%; varicella 3.1%; gastritis 3.1%
Okada, 2007 [xlvi]	Prospective	11	750-1000	1	Not reported	36.4%	9.1%	3.2±3.1 mg/kg/month	Gastrointestinal AE 18.2%; alopecia 9.1%
Fujinaga, 2007 [xlvii]	Prospective	12	1220±95	0.9 (0.5-6.5)	0.6±0.9	25% at 6 months	Not reported	0.21±0.11	None
Afzal, 2007 [xlviii]	Retrospective	42	26.5 (16.6-31.3) mg/kg	1.2 (0.5-6.8)	2.2 (1.4, 2.9)	78.6%	11.9%	0.3 (0.3, 0.4)	Abdominal pain 21.4%; infections 9.5%
Fujinaga, 2009 [xlix]	Retrospective	26	34±6 mg/kg	1.6 (0.6-6.5)	0.8±1.2	Not reported	Not reported	0.17±0.11	Anemia and herpes labialis in 3.8% each
Baudouin, 2012 [l][$]	Prospective	23	1200	1	Not reported	26.1%	Not reported	264 (196–306) mg/m^2/month[^]	Gastrointestinal AE or infections in 26.1%; leukopenia or anemia in 30.4%
Hasan, 2013 [li]	Retrospective	61	1200	3.2 (1.7-4.7)	0.5 (0–0.87)[^]	51%	38%	Withdrawn in 56%	Gastrointestinal AE 13%; leukopenia or infections 11%; arthralgia 3%

Banerjee, 2013 [lii]	Retrospective	46	20-30 mg/g	3.6±1.8	Not reported	57%	No response in 33.3%	Reduced in 70%	Gastrointestinal AE 7.4%; neutropenia and elevated transaminases in 3.1% each
Jellouli, 2016 [liii]	Retrospective	30	1200	Not reported	0.45	Not reported	Not reported	0.2	Not reported
Basu, 2017 [liv]	Retrospective	130	1200	2.5	0.9±0.4	13.1% (at 1 yr)	6.1%	108.8±35.7 mg/kg	Gastrointestinal AE 3.8%; infections 6.2%; other minor 1.5%
Karunamoorthy, 2019 [lv]	Retrospective	87	28.5 mg/kg	3.3 (1.3-6.5)	Not reported	72.4%	17.2%	0.35^	Infections 12%; diarrhea 6%; leukopenia 3%; gastritis 2%

$*Single limb Bayesian randomized controlled trial; ^Reported only for patients with response*

Supplementary Table IX Randomized Controlled Trials (RCT) on Mycophenolate Mofetil (MMF) in Steroid Sensitive Nephrotic Syndrome

Author, yr [ref]	*Type of RCT*	*N*	*MMF dose, mg/m² per day*	*Comparator*	*Follow up, yr*	*Outcomes at 12-24 months*				*Adverse events (AE)*
						Relapses, n or rate (95% CI)	*Proportion with relapses*	*Frequent relapses*	*Cumulative predniso(lo)ne, mg/kg per day*	
Dorresteijn [lvi]	Open label	24	1200	Cyclosporine 4-5 mg/kg/day	1	0.83±1.3 *vs.* 0.08±0.3	41.7% *vs.* 8.3%	8.3% vs. 0%	0.13±0.16 *vs.* 0.08±0.12	First 3 studies: Hypertension 8.3% *vs.* 29.2%; hypertrichosis 6.9% *vs.* 40.3%; leukopenia 2.4% *vs.* 4.8%; gum hypertrophy 0% *vs.* 20.8%; reduced eGFR 0% *vs.* 8.3%; diarrhea 13.3% *vs.* 0%
Gellermann [lvii]	Cross-over, open label	60	1000; titrated to level	Cyclosporine 150 mg/m² per day	2	1.1±2 *vs.* 0.4±0.7*	42.9% *vs.* 30%	Not reported	1.83 *vs.* 0.99 g/m²	
Uddin [lviii]	Open label	60	800-1200	Cyclosporine 4-5 mg/kg/day	0.5	3±2.9 *vs.* 1.4±2.6	Not reported	Not reported	Not reported	
Wang [lix]	Not RCT	72	24.6±3.1 mg/kg/day	Tacrolimus 0.08±0.02 mg/kg/day	1	1.43 *vs.* 0.83	~58% *vs.* ~48%	12.2% *vs.* 0%	0.16±0.02 *vs.* 0.17±0.03	Infections 11.8% *vs.* 7.9%; gastrointestinal AE 11.8% *vs.* 2.6%; leukopenia 2.7% *vs.* 2.6%
Sinha [xlv]	Open label	149	750-1000	Levamisole 2-2.5 mg/kg on alternate days	1	1.1 (0.3, 1.3) *vs.* 1.3 (1.1, 1.7)	65.8% *vs.* 65.7%	16.4% *vs.* 14.5%	0.2 (0.1, 0.4) *vs.* 0.3 (0.2, 0.4)	Increased aminotransferases 2.6% *vs.* 2.7%; leukopenia 1.3% *vs.* none

AE adverse event; eGFR estimated glomerular filtration rate
**P <0.05; one Bayesian RCT is included in Web Table IX, since it lacked a comparator limb*

Supplementary Table X Determinants of Response to Therapy with Cyclophosphamide

Author, yr	*Cyclophosphamide cumulative dose*	*N*	*Age, yr*	*Follow up, yr*	*Proportion (%) in remission at 1, 2, 5 & 10 yr*[^]	*Factors associated with prolonged remission*
Latta 2001 [lx]	105-588 mg/kg	1504; 38 studies	NA	NA	Frequent relapses/dependence: NA/NA; 72/40; 36/24; NA/NA	Frequent relapses*; cumulative dose of cyclophosphamide
Vester 2003 [lxi]	165±33 mg/kg	106	7.3±3.8	NA	44; 34; 24; 24	Age >5.5-yr; frequent relapses*; cumulative dose >5 g/m^2; leukopenia
Kyrieleis 2007 [lxii]	~168 mg/kg	80	~4 (2-15)	6 (2-27)	NA; 35; ~48; ~60	Age >3-yr
Zagury 2011 [lxiii]	175 mg/kg	108	4.9	9.5 (5-29)	NA; 34; 25; 22	Relapse threshold <1.4 mg/kg; age >7-yr (univariate analysis)
Cammas 2011 [lxiv]	168 (157-197) mg/kg	143	7.9 (4.6-11.2)	7.8 (4-11.8)	44; 27; 13; 11[^1]	Age >5-yr; cumulative dose >170 mg/kg
Azib 2011 [lxv][#]	160 (149–170) mg/kg	90	5.3 (3.2–9.1)	5.5 (3.2-8.5)	57, 42, 31, NA[^2]	Age >7.5-yr
Berkane 2018 [lxvi]	168 mg/kg	50	8	1.6	52; 48; NA; NA	Age>8-yr; frequent relapses*

NA not available
**versus steroid dependence*
[^]Median time to relapse not reported, except [^1]10 months and [^2]0.8 (0.4-1.5) years
[#]All patients were steroid dependent

Supplementary Table XI Controlled Studies Examining Comparative Efficacy of Rituximab in Steroid Sensitive Nephrotic Syndrome

Author, yr	*Rituximab mg/m²; n*	*Control*	*N*	*Follow up, yr*	*Outcomes*				
					Relapse rate (RR)	*Proportion with relapse (HR; 95% CI)*	*Time to relapse, mo*	*% off steroids*	*% off all agents*
Randomized clinical trials									
Iijima 2014 [lxvii]	375, 4	Placebo	24; 24	1	1.5 *vs.* 4.2 per p-yr (0·37; 0·2, 0·6)	71% *vs.* 96% (0.27; 0.1, 0.5)	8.9 *vs.* 3.4	88% *vs.* 79%	NA
Boumediene 2018 [lxviii]	375, 2[#1]	Placebo[#1]	10; 13	0.5	NA	10% *vs.* 100%	NA	NA	NA
Ahn 2018 [lxix]	375, 1[#1]	None[#1]	40; 21	0.5	3.4 *vs.* 9.4 per p-yr	26% *vs.* 69%	9 *vs.* 2.9	NA	NA
Ravani 2020 [lxx]	375, 1[#]	None[#]	15; 15	1	NA	13% *vs.* 7%	NA *vs.* 1.5	NA	NA
Ravani 2015 [lxxi]	375, 1[#]	Prednisone[#]	15; 15	0.25 (1)	NA	20% *vs.* 93%[$] (0.02; 0.01, 0.15)	18 *vs.* NA	NA	NA
Ravani 2011 [lxxii]	375, 1-2	CNI alone	27; 27	0.25 (1)	NA	19% *vs.* 48% at 3-months	NA	78% *vs.* 7.4%	63% *vs.* 3.7%
Basu 2018 [lxxiii]	375, 2	Tacrolimus	60; 60	1	NA	10% *vs.* 37%	10 *vs.* 7	93% *vs.* 79%	NA
Single arm clinical trials									
Ruggenenti 2014 [lxxiv]	375, 1	None	30[^]	1	0.5 (0-1)	70% in children	7.5	NA	60%
Non-randomized prospective (P) or retrospective (R) comparisons									
Kari 2020 (P) [lxxv]	375, 2	Cyclophosphamide	19; 27	1	NA	16% *vs.* 41% (0.36; 0.1, 1.5)	NA[$]	74% *vs.* 30%	NA
Webb 2016 (R) [lxxvi]	750, 2	Cyclophosphamide	42; 79	≥1	NA	50% *vs.* 60%[$]	14 *vs.* 7	NA	69% *vs.* 84%

Sinha 2012 (R) [lxxvii]	375, 2-3	Tacrolimus	10; 13	1	0.8±1.0 *vs.* 0.9±1.1	50% *vs.* 54%[$]	8.5 *vs.* 9.8	80% *vs.* 46%	80% *vs.* 46%
Ongoing randomized clinical trials									
Nagano [lxxviii]	375, 2	Placebo	20; 20	1	Awaited; JMA-IIA00380				
Ravani [lxxix]	375, 1[#1]	Ofatumumab 1500 mg/m^2, 1[#1]	70; 70	2	Awaited; NCT02394119; Eudra-CT 2015-000624-28				
Mathew	375, 2	Tacrolimus	21; 20	1	Awaited; CTRI/2018/11/016342				

NA not available; p-yr person-year; yr year

[#]Steroids and [#1]CNI tapered; ^Includes 10 children; [$]Based on Kaplan Meier estimates of relapse-free survival at 1-yr

Supplementary Table XII Strategies to Maintain Remission Following Rituximab Administration

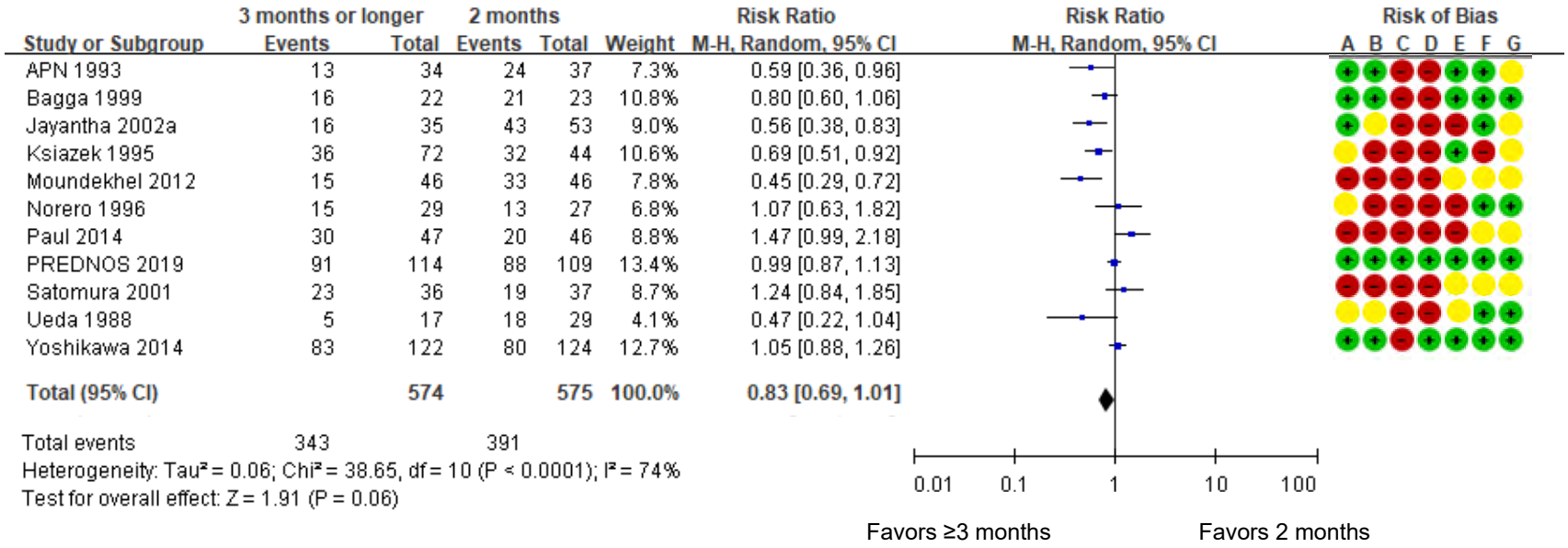
Author, year	*RTX* doses*	*Immunosuppression*	*N*	*Follow up, yr*	*Results*
Maintenance immunosuppression (mIS)					
Ito 2011 [lxxx]	1	MMF *vs*. none	9 *vs*. 7	1 yr	MMF therapy led to fewer relapses (0.4 *vs.* 2.3) and relapsers (33% *vs*. 86%) at 1-yr
Fujinaga 2013 [lxxxi]	1	CsA *vs*. MMF	13 *vs*. 16	1.5 yr	CsA *vs.* MMF led to fewer relapses (0.6±1.4 *vs.* 1.0±0.9); lower rates of relapse (25% *vs*. 45%) and lower treatment failure (15% *vs*. 44%); steroid sparing
Hourinouchi 2018 [lxxxii]	4	MMF *vs*. placebo	40 *vs*.40	1.4 yr	Awaited; UMIN000014347
Number of doses					
Hogan 2019 [lxxxiii]	1[*1] *vs*. 1 *vs*. 2	None	8 *vs*. 35 *vs*. 18	≥1 yr	Proportions in sustained remission at 1-yr higher by dose: 50 (58–77) % for 100 mg/m^2; 59 (42-76) % for 375 mg/m^2 and 72 (46-87) % for 750 mg/m^2 Low *vs.* high dose associated with risk of relapse: HR 5.0 (1.2, 21.6)
Maxted 2019 [lxxxiv]	1 *vs*. 2-3 *vs*. 4[*2]	Details not available	40 *vs*. 5 *vs*. 15	≥1 yr	1, 2-3 or 4 dose equivalents: Similar proportions in sustained remission at 1-yr (47%, 71%, 53%); similar time to relapse (334, >720, 344 days)
Number of doses and maintenance immunosuppression (mIS)					
Chan 2020 [lxxxv]	1 *vs*. 2 *vs*. 3-4	Prednisone, CNI or MMF [Continued *vs*. stopped]	191 *vs*. 208 *vs*. 112	≥0.5 yr	Time to relapse: *(i)* Similar for 1, 2 or 3-4 doses (11.8, 11.9, 13 months); *(ii)* similar among patients on mIS (11.8, 11.9, 13 months); *(iii)* lower for 1 vs. 2 or 3-4 doses if not given mIS (8.5, 12.7, 14.3 months); adjusted HR 0.5 & 0.6 (0.3-0.9)
Sequential administration of doses					
Takei 2013 [lxxxvi]	1 q 6 mo; 2 doses	Prednisone; CNI, MMF or mizoribine	25 adults^	1 yr	Before *vs*. after: Fewer relapses (62 *vs*. 4) and reduced prednisone (8.2±3.4 vs. 3.3±2.3 g/yr); 80% off prednisone and mIS; increased serum IgG (P=0.0005)
Miyabe 2016 [lxxxvii; lxxxviii]	1 q 6 mo; 4 doses	Prednisone; CNI, MMF or mizoribine	25^& 54^adults	2 yr	Before *vs*. after: Fewer relapses and reduced prednisone; all off prednisone and mIS; increased IgG; improved bone mineral density and blood pressure
Iwabuchi 2018 [lxxxix]	1 q 6 mo;4 doses	Prednisone; CNI, MMF or mizoribine	32 children & 19 adults^	2 yr	In children *vs*. adults: Few relapses and minimal prednisone dose (P <0.001); similar frequency of adverse reactions (21% *vs*.20%)
Papakrivopoulou 2016 [xc]	1 q 6 mo; 2-3 doses	Prednisone off by 3-mo; CNI tapered at >1-yr	15 adults	1.7 yr	Before *vs.* after: Fewer relapses (P <0.001); median remission 25 months; IgG levels unchanged

Taguchi 2020 [xci]	1 q 6 mo; 2-4 doses		13 adults	2 (1-5) yr	Before *vs.* after: Reduced relapses, and prednisone and cyclosporine dosage
Kim 2018 [xcii]	At B cell recovery[@1]	Details NA	12 children	2±1 yr	Before *vs.* after: Fewer relapses and off mIS (P <0.01)
Sellier-Leclerc 2012 [xciii]	At B cell recovery[@2]	MMF off; prednisone and CNI off by 3-mo	30 children	≥2 yr	Sustained remission in 63% at 3.2±0.1 yr; 37% relapsed 4.3 months after B cell recovery; 100% off mIS; transient adverse effects

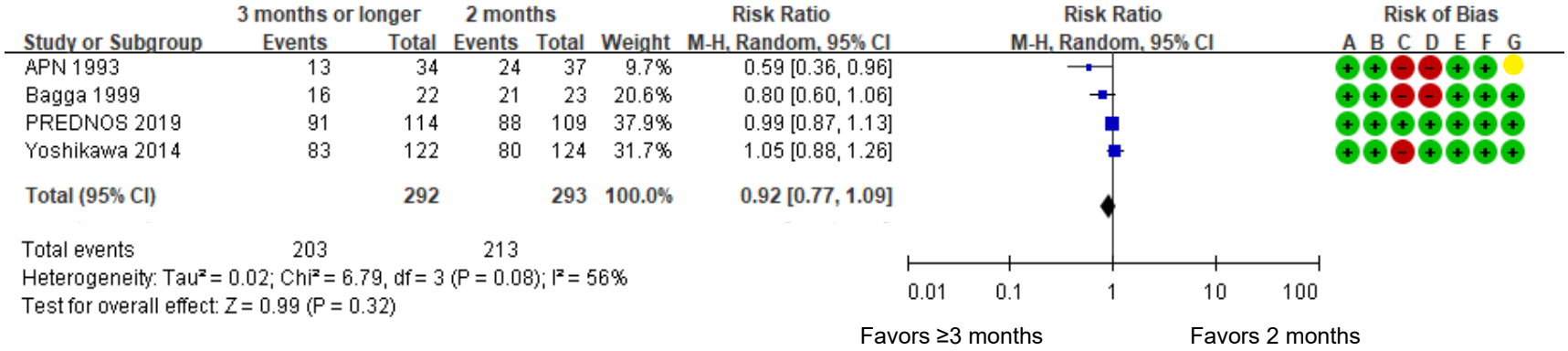
CNI calcineurin inhibitor; HR hazards ratio; IgG immunoglobulin G; MMF mycophenolate mofetil; mo months; NA not available; yr year
**Each dose was 375 mg/m^2 except[*1] where it was 100 mg/m^2 or [*2]750 mg/m^2 x 2 or 375 mg/m^2 x 4 doses*
[^]Overlap of patients between studies is unclear
[@]Total doses and frequency were [1]3.9±1.6 doses q 6±2 months and [2]5±1.4 doses over 15 months

Supplementary Figure I Meta-analyses of Randomized Controlled Trials on Prednisone Therapy for First Episode of Nephrotic Syndrome

Comparison 1.1.1 3-months or longer versus 2-months: Occurrence of relapse (all studies)



Comparison 1.1.2 3-months or longer versus 2-months: Occurrence of relapse in studies at low risk of bias

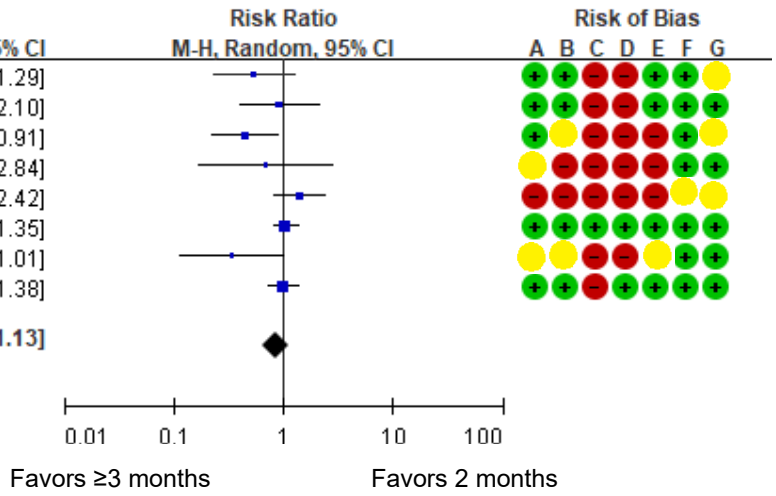


Comparison 1.2.1 3-months or longer versus 2-months: Occurrence of frequent relapses (all studies)

Study or Subgroup	3 months or longer Events	Total	2 months Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
APN 1993	6	34	12	37	7.9%	0.54 [0.23, 1.29]
Bagga 1999	7	22	8	23	8.4%	0.91 [0.40, 2.10]
Jayantha 2002a	8	48	26	70	10.7%	0.45 [0.22, 0.91]
Norero 1996	3	29	4	27	3.5%	0.70 [0.17, 2.84]
Paul 2014	20	47	14	46	14.6%	1.40 [0.81, 2.42]
PREDNOS 2019	60	114	55	109	26.4%	1.04 [0.81, 1.35]
Ueda 1988	3	17	15	29	5.5%	0.34 [0.12, 1.01]
Yoshikawa 2014	45	122	46	124	23.1%	0.99 [0.72, 1.38]
Total (95% CI)		**433**		**465**	**100.0%**	**0.86 [0.65, 1.13]**
Total events	152		180			

Heterogeneity: Tau² = 0.06; Chi² = 12.50, df = 7 (P = 0.09); I² = 44%

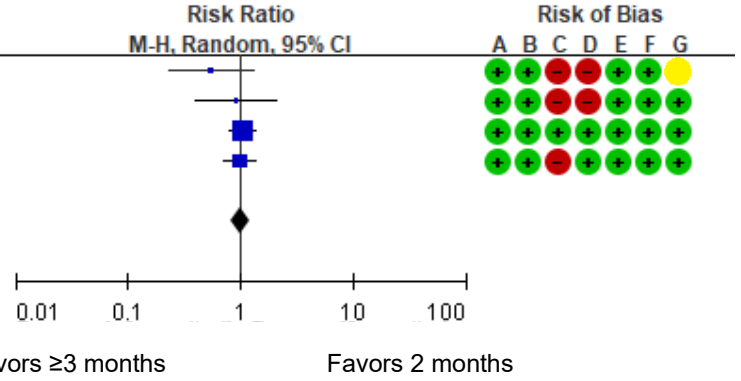
Test for overall effect: Z = 1.09 (P = 0.28)



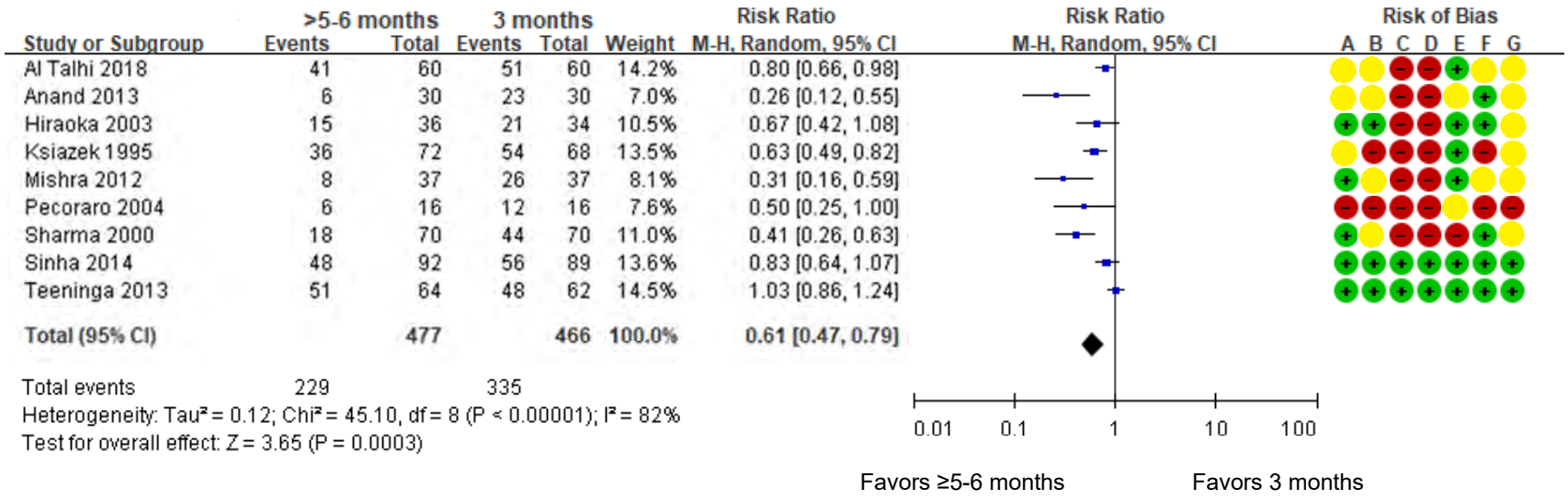
Comparison 1.2.2 3-months or longer versus 2-months: Occurrence of frequent relapses in studies at low risk of bias

Study or Subgroup	3 months or longer Events	Total	2 months Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
APN 1993	6	34	12	37	4.9%	0.54 [0.23, 1.29]
Bagga 1999	7	22	8	23	5.3%	0.91 [0.40, 2.10]
PREDNOS 2019	60	114	55	109	55.8%	1.04 [0.81, 1.35]
Yoshikawa 2014	45	122	46	124	34.1%	0.99 [0.72, 1.38]
Total (95% CI)		**292**		**293**	**100.0%**	**0.99 [0.82, 1.19]**
Total events	118		121			

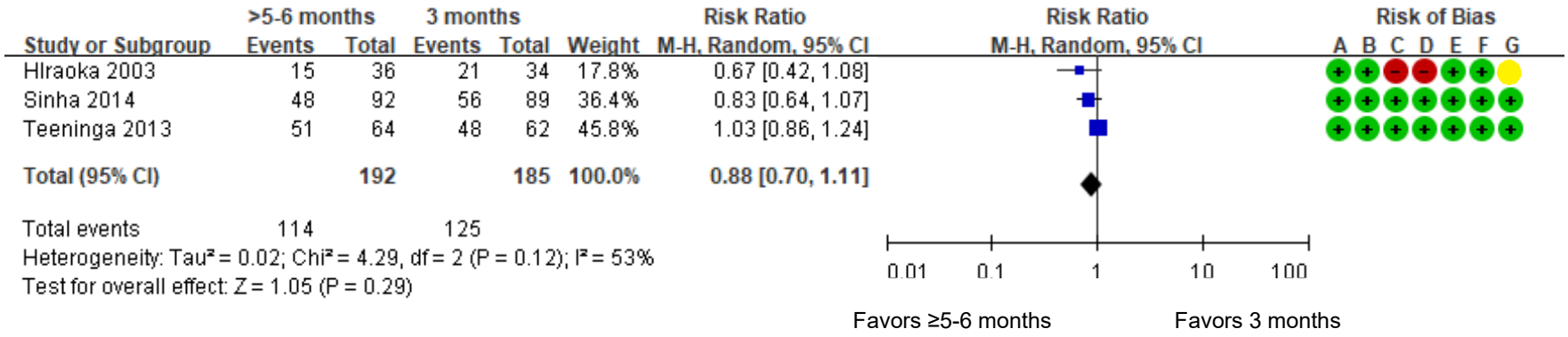
Heterogeneity: Tau² = 0.00; Chi² = 2.08, df = 3 (P = 0.56); I² = 0%
Test for overall effect: Z = 0.13 (P = 0.90)



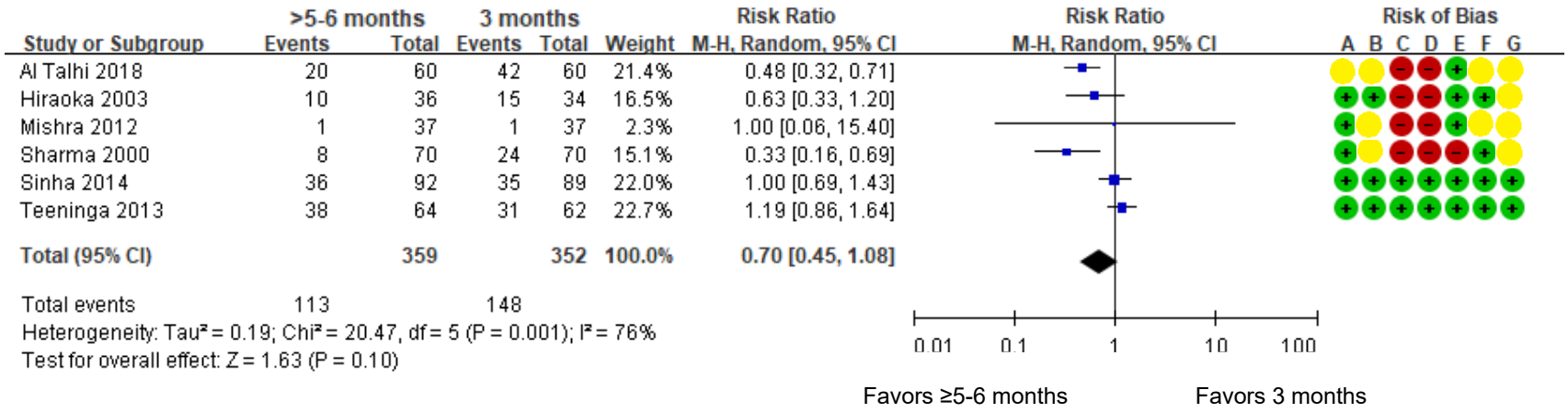
Comparison 2.1.1 5-6 months or longer versus 3 months: Occurrence of relapse (all studies)



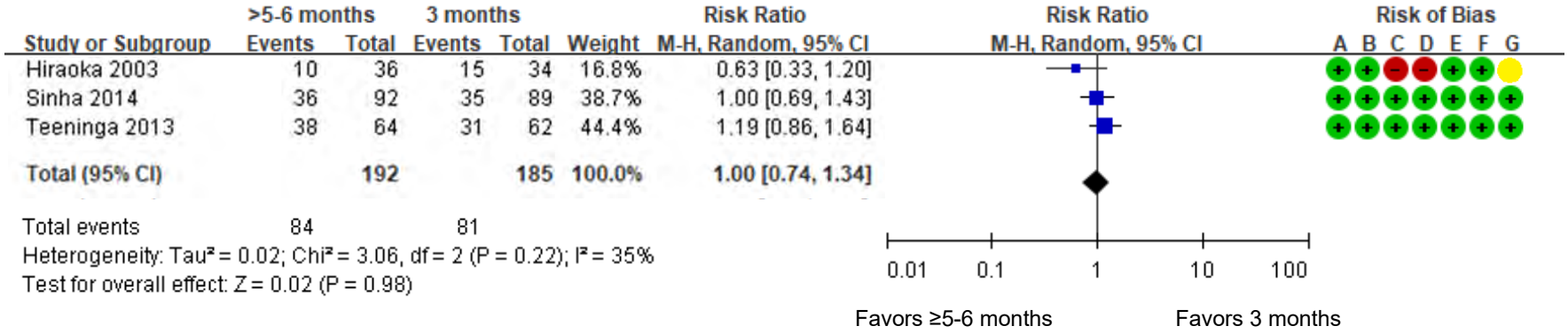
Comparison 2.1.2 5-6 months or longer versus 3-months: Occurrence of relapse in studies at low risk of bias



Comparison 2.2.1 5-6 months or longer versus 3-months: Occurrence of frequent relapses (all studies)



Comparison 2.2.2 5-6 months or longer versus 3-months: Occurrence of frequent relapses in studies at low risk of bias



Legend for risk of bias assessment

Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

References for Supplementary material

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OBITUARY

Dr. Youraj Chandra Mathur was born in 1934. Dr YC Mathur grew up at an age when medicare was not even in its infancy in India. Dr Mathur was particularly concerned about child mortality in his childhood having lost his brother and a few playmates at a very tender age. This inspired him to be a doctor, and more importantly a child specialist. He passed his MBBS in 1957, DCH in 1958 and MD in 1961. He joined AP Medical Services in 1958 and worked as Assistant Professor in Niloufer Hospital since 1962. Dr YC Mathur pioneered the concept of Social Pediatrics to offer a package of comprehensive healthcare. He started the Hyderabad mix (Vegetable Protein Mix) in association with NIN to bridge up the calorie gap- in Rural areas and Urban Slums, which is emulated now in various National and International Programs. He held the post of President of the Indian Academy of Pediatrics (National Executive). He had been a mentor to many senior IAP members in Telangana, Andhra Pradesh, and all over India. In his demise the Indian Academy of Pediatrics has lost one of its illustrious sons. The IAP conveys its condolences to the family and pays homage to the departed soul.

Varicella Outbreak in Children from Silvassa, Dadra and Nagar Haveli, India

Study describes epidemiological and laboratory findings of the fever with skin-rash cases (*n*=247) reported from Dadra and Nagar Haveli during 2018-19. For laboratory diagnosis, 33 sera and 5 blister swabs were obtained from 36 suspected cases. Varicella-zoster-virus DNA PCR and IgM EIA confirmed 33 cases and sequencing revealed circulation of clade-1 viruses.

Keywords: *Exanthematous illness, Field investigation, Laboratory diagnosis*.

Varicella infection is caused due to varicella zoster virus (VZV) that belongs to family *Herpesviridae* and genus *Varicellovirus*. Primary infection of VZV is referred to as 'chickenpox' and subsequent VZV reactivation as 'herpes zoster or shingles' [1]. Varicella outbreaks are frequently reported from various regions of India [2-9]. Varicella vaccine is not included in India's Universal Immunization Program (UIP) however; Indian Academy of Pediatrics recommends two doses of the vaccine (http://www.iapindia.org) at 15 months, 3-6 months apart.

Dadra and Nagar Haveli (DNH) consists of 70 different villages situated on the western coast of India. During 2018-19, in Silvassa block, cases of fever with skin rash were reported amongst 14 villages. A standard case definition was followed for suspected varicella [10], and the case details were recorded in a standard data sheet (i.e. patient details like age, gender, place, date of clinical onset, type of rashes etc.) Overall, 247 (male; female, 1.22:1) fever with skin rash cases (including one pregnant woman) were recorded from 14 villages, without any mortality.

Thirty three and 17 serum samples were collected from the skin-rash cases and their close contacts, respectively. Both the serum samples and blister swabs were available from two suspected cases, whereas only blister swabs were available from three suspected cases. All these sera were subjected to anti-VZV IgM and IgG antibody detection [10]. Four blister swabs and one blister swab, respectively collected from Velugam and Surangi villages were processed and subjected to VZV DNA PCR [10]. PCR positive blister swabs were used for virus isolation in Vero cells. PCR amplicons were sequenced using forward and reverse primers, and the consensus sequence was submitted to GenBank (MK959623 to MK959627). All data were analyzed using Epi Info software version 7.2. Descriptive statistics were reported as mean and standard deviation (SD).

Between November, 2018 and April, 2019, 247 cases of fever with skin-rash were reported from 14 villages of Silvassa block of DNH with male-female ratio of 1.22:1. The mean (SD) age was 8.65 (6.48) year with 92% (228) patients younger than 18 years. The distribution of fever with skin-rash cases is presented in **Fig. 1**. Case follow-up was done up to 25 weeks and none of the cases required hospitalization. The initial symptoms of vomiting (*n*=31), appetite loss (*n*=203), muscle pain (*n*=41) and headache (*n*=179) was followed by fever and skin rashes (247). None of the cases reported pneumonia or other complications and all the cases recovered without any further clinical co-morbidities. Interestingly, clinical symptoms were not reported in any of their close contacts (i.e. 12 children and 5 adults).

Thirty three skin rash cases were confirmed by anti-VZV IgM EIA and VZV DNA PCR and in 3 cases anti-VZV IgG EIA was positive. Serological and molecular analysis confirmed varicella in 92% of them. Twenty eight laboratory confirmed varicella cases (VZV IgM) had median (IQR) onset of 10 days (8-12) (33 out of 36). Thirty six suspected varicella cases included 35 children (and 1, 24 yrs adult) of which 90% were (9 out of 10) females and 92.3% (24 out of 26) were males. Of the 17 contacts, none showed laboratory confirmed varicella but 15 contacts showed anti-VZV IgG antibodies, indicating past exposure. The PCR amplicons (*n*=5) were sequenced and a consensus gene fragment was used for sequence similarity search using BLAST (*https://blast.ncbi.nlm.nih.gov/Blast.cgi*), which indicated the presence of VZV clade-1. Passaging of blister swabs in vero cells failed to show cell cytopathic effect.

Previously, we reported varicella outbreaks in two villages from other part of Dadar and Nagar Haveli, where a circulation of clade-1 VZV was documented [10]. Present report confirms circulation of a similar VZV clade.

Absence of health awareness and delayed isolation of cases at home may have resulted in clusters at both villages. Interestingly, majority of cases had travel history to nearby villages and may be additional source of transmission; however, this could not be investigated. In addition, not all cases were referred for laboratory investigations.

Varicella is vaccine preventable disease but yet to garner attention in India. Study emphasizes the need for more

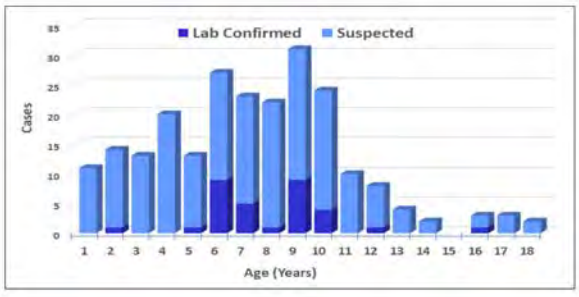

Fig. 1 Age-wise distribution of suspected and laboratory confirmed varicella infection in children below 18 years (*n*=230).

investigations on skin rash cases to detect etiology, so as to have better epidemiological picture of varicella in the country.

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Multicystic Hepatic Lesion: An Unusual Presentation of Extra-Pulmonary Tuberculosis in a Child

Tuberculosis of the liver is a rare form of extra pulmonary tuber-culosis, and is seen more commonly in immunocompromised patients or in association with disseminated tuberculosis. Liver involvement without involvement of lung or other organs is rare. Nodular form of liver tuberculosis presenting as abscess is uncommon, and is commonly mistaken for pyogenic/amebic liver abscess or malignancy. Definitive diagnosis requires detection of tubercular bacillus in pus or liver biopsy [1].

A 12-year-old girl presented with non-localized upper abdominal pain for 3 months, with history of fever off-and-on and weight loss of 4 kg in two months. There was no history of previous hospitalization or contact with tuberculosis. Immunization was complete as per national immunization schedule; however, BCG scar was absent. On examination, child was stunted and wasted (weight for age at –2.04 *z*-score, height for age at –2.54 *z*-score as per IAP charts). General physical examination revealed severe pallor, angular cheilitis, mucositis and knuckle hyperpigmentation, with no lymphadenopathy. On systemic examination, there was hepatomegaly with other systems being unremarkable. Chest radiograph was normal. Ultrasonography abdomen revealed a large heterogenous solid cystic mass lesion involving the segment VIII and IV of liver, extending till the subcapsular regions. A possibility of hydatid cysts, multiple pyogenic abscesses and fungal abscesses was kept. On laboratory evaluation, hemogram was performed: Hemoglobin 4.4 gm/dL, total leucocyte count 17200/mm^3 (lymphocytes 18%, neutrophils 78%) and peripheral smear revealed dimorphic blood picture with microcytic hypochromic and macrocytic normochromic red cells. ESR was raised (60 mm/h); liver and renal function tests were normal. Serum iron levels (40 mcg/dL) and serum B12 levels (160 pg/mL) were both low. Mantoux test and gastric aspirate for cartridge based nucleic acid amplification test (CBNAAT) were negative. Stool microscopy did not reveal cyst or ova and HIV test and immunodeficiency work up was negative. Computed tomography (CT) scan of abdomen revealed a large ill-defined heterogeneously hypodense mass lesion involving the left and right lobes (segment VIII and IV) of liver, faintly hyperdense internal septations could be seen (**Fig. 1**). A possibility of hydatid cysts and malignancy was kept. Hydatid serology was negative. CT-guided tru-cut needle biopsy was planned, for which the child was referred to a higher centre. Liver biopsy showed multiple epithelioid cell granulomas, positive for acid-fast bacilli on Ziehl Neelson (ZN) stain. A diagnosis of tuberculosis was made and child was started on directly observed treatment, short-course (DOTS) therapy for tuberculosis. On follow up after 2 months, child started gaining weight and repeat ultrasonography showed decreasing size of liver abscesses.

Primary hepatic tuberculosis without pulmonary or miliary tuberculosis is an uncommon diagnosis. The diagnosis is frequently missed due to lack of suspicion and can mimic other etiologies like bacterial, amebic or fungal liver abscess [2]. In a study from South Africa, in 296 patients with hepatic tuberculosis, tubercular abscess accounted for only 0.54% cases [3]. In an Indian study of 242 immunocompetent tuberculosis patients, 38 had liver involvement, of which 10 had tubercular liver abscess [4]. Patients usually present with fever, abdominal pain, anorexia, hepatomegaly and loss of weight with jaundice being an uncommon presentation. Right lobe of liver has been found to be more commonly involved (82.5%) [1].

Radiological findings are variable and insufficient for diagnosis. Majority of the cases have shown heterogenous, anechoic or hypoechoic lesions with irregular margins; however, some reports have described a hyperechoic mass [5]. Amebic or pyogenic liver abscess or hepatocellular carcinoma are the differential diagnosis. Definitive diagnosis can be made by detection of tubercular bacilli in pus or liver biopsy stained by ZN stain [1]. Although culture is the gold standard, but it requires long incubation period. Polymerase chain reaction has a sensitivity of 92.4% and specificity of 98%, and should be performed for rapid diagnosis [6].

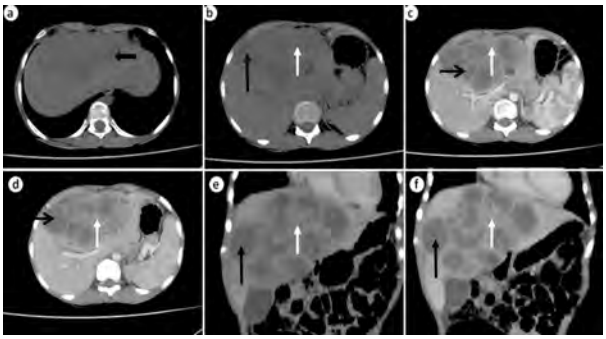

Fig. 1 (*a*) Axial plain CT images reveal a large ill-defined heterogeneously hypodense mass lesion involving left and right lobes (segment VIII and IV) of liver; (*b*) Cystic appearing areas (black arrow) as well as solid slightly hyperdense areas (white arrow) seen within the lesion. Contrast enhanced CT scan shows axial images: late arterial phase (*c*) and porto venous phase (*d*), composed of solid and cystic areas. Cystic areas (black arrow) show no significant enhancement whereas solid areas show mild progressive enhancement (white arrow); Contrast enhanced CT scan shows coronal images of the lesion. (*e*) late arterial phase, (*f*) porto venous phase. Lesion is composed of solid and cystic areas. The cystic areas (black arrow) show no significant enhancement whereas solid areas show mild progressive enhancement (white arrow).

We report this case to highlight a rare manifestation of a common disease. A high index of suspicion may help in timely diagnosis and avoid unnecessary investigations or surgical intervention.

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Benign Recurrent Intrahepatic Cholestasis - Unravelleing the Paradox

Benign recurrent intrahepatic cholestasis (BRIC) is a rare autosomal recessive bile salt transport disorder, characterized by recurrent episodes of pruritus, cholestatic jaundice with normal or low gamma glutamyl transpeptidase (ggt). Bric and progressive familial intrahepatic cholestasis (PFIC) represent two extremes of a continuous spectrum of genetic intrahepatic cholestatic disorders. The exact prevalence of BRIC still remains unknown. BRIC can present at any age but usually before the second decade [1] with disturbing pruritus as the primary symptom. Very few case reports have been published in Indian literature [2,3]. We report the clinico-laboratory profile and follow up of seven patients with BRIC seen over a period 20 years (2000-2020).

Patient 1: A 16 year old adolescent boy, 1st born to 3^0 consanguineous parents, presented with 2 weeks history of severe pruritus associated with mild jaundice. His first cousin died of cholestatic liver disease at the age of 7 years. On examination, he was well nourished with icterus and scratch marks on his skin. There was no hepatosplenomegaly. Complete hemogram and renal profile were normal. His total serum bilirubin was 5.1 (direct:4.1) mg/dL, alamine aminotransferase (ALT) was 133U/L, aspartate aminotransferase (AST) was 143U/L, γ-glutamyl transferase-20 U/L, serum alkaline phosphatase-268 U/L, cholesterol -145 mg/dL and serum bile acids 491 µmol/L (0.5-10). Prothrombin time was normal. USG abdomen showed normal liver echogenecity and intrahepatic radicles were not dilated. Liver biopsy was deferred due to refusal of consent. In view of the strong positive family history of cholestatic liver disease, gene testing was done that showed a single heterozygous missense mutation [c.1244A>G] in exon 13 of *ATP8B1* gene confirming BRIC type 1. He was treated with rifampicin for 3 weeks, and at 5 months follow up, all his laboratory parameters were normal.

Patient 2: A 16-year-old boy presented with intense pruritus and jaundice for 20 days. There was history of two stereotypical episodes of pruritus associated with cholestatic jaundice in the last 1 year. During the icterus free intervals, he was normal. He was second born to second degree consanguineous parents, and his elder sister had been diagnosed as BRIC type 1. On examination, he was well nourished with deep icterus and scratch marks on his skin. There was no hepatosplenomegaly. Investigations revealed normal hemogram and renal profile. Total bilirubin was 31 (direct: 25.3) mg/dL. ALT, AST and total protein were normal. Serum bile acids were 350 µmol/L and GGT was 12 U/L. Magnetic resonance cholangiopancreatography (MRCP) showed mild hepatomegaly without intra or extra hepatic biliary dilatation. Liver biopsy showed marked canalicular cholestasis, mild lobular inflammation, with intact interlobular bile ducts and no fibrosis. He was treated with ursodexycholic acid (UDCA) and rifampicin, and at 4 weeks his jaundice had cleared. Genetic testing showed a homozygous missense mutation [c.922G>A] in exon 10 of *ATP8B1* gene confirming BRIC type 1. He is under follow up for the last three years, and is doing well without any worsening.

In the remaining five patients (3 girls) with BRIC diagnosed histologically, the median age at onset of symptoms was 11 (range: 8-18) years. The cholestatic episodes varied with an average of 1 to 3 per year and the reported asymptomatic periods were lasting for a maximum period of 3 years. There was history of consanguinity in 80%, of which second and third degree consanguinity was seen in 50% each. One child was adopted. Liver histology done in all five patients showed intrahepatic cholestasis with intact interlobular bileducts and no fibrosis. All were treated with UDCA, rifampicin and cholestyramine in varying combinations. Over these 20 years, 2 girls got married and both had pruritus during pregnancy. Another boy diagnosed with BRIC at 8 years was given complementary and alternative medicine for refractory pruritus at 17 years, that worsened his liver function following which, the jaundice deepened, bilirubin rose to 40 mg/dL and his INR reached 4. He underwent 3 cycles of plasmapheresis, but succumbed to the illness prior to liver transplant.

A typical episode of BRIC usually starts with pruritus which increases in intensity and impairs the quality of life followed by jaundice as seen in our series [4]. The symptoms may persist from 2 weeks to 18 months before spontaneous resolution and asymptomatic period may vary from 1 month - 33 years [3]. Low GGT, a hallmark biochemical finding in this metabolic disorder in spite of clinical and biochemical stasis excludes all the intra- and extra- hepatic causes of cholestasis except bile acid synthesis defect (BASD). However, in BASD, itching is not common and low GGT occurs with normal level of bile acids. The characteristic changes seen in liver biopsy in BRIC are the intracanalicular cholestasis with lobular inflammation without fibrosis, which was seen in all the 6 patients, thus satisfying the diagnostic criteria [5]. PFIC1 and BRIC 1 share the same genotype but have different phenotypes, the former being universally progressive. *ATP8B1* gene is a translocator present on canalicular membrane of hepatocytes and mutation leads to membrane instability and decreased function of bile salt export pump thereby resulting in cholestasis. Missense mutations are most common in BRIC type 1 [6] (seen in patient 1 and 2) and can either be homozygous (patient 2) or compound heterozygous mutations (patient 1). However, Lee, et al. [7] have reported a similar phenomenon of heterozygous frame shift mutation only on one allele of *ATP8B1* gene. Cholestyramine, UDCA and rifampicin have been used in various combinations for the treatment of pruritus and in our experience, neither cholestyramine nor UDCA worked well while rifampicin alone gave a sustained relief in one patient. Rifampicin, though considered as hepatotoxic, works well in BRIC by activating transcription of CYP3A4, thereby stimulating hydroxylation of bile salts and excretion at the basolateral membrane, thereby relieving pruritus [8]. Endo-scopic nasobiliary drainage [9], biliary diversion procedures [10], plasmapheresis and liver transplant have been suggested for refractory pruritus.

This case series highlights the paradoxical perceptions in diagnosis and management of BRIC, namely low GGT in spite of cholestasis and use of rifampicin-a hepatotoxic drug, inspite of underlying liver disease.

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Diabetes Mellitus Due to Wolfram Syndrome Type 1 (DIDMOAD)

Wolfram syndrome (WFS) type 1 is a monogenic disorder with autosomal recessive inheritance caused by mutations in *WFS1*, a gene (location 4p16.1) associated with endoplasmic reticulum function in neuronal and endocrine cells [1]. WFS is also known as DIDMOAD syndrome and is characterised by Diabetes insidipus (DI), Diabetes mellitus (DM), Optic atrophy (OA), and Deafness (D). Here, we report 5 unrelated Indian children presenting to us over the last 2 years with a referral diagnosis of type 1 diabetes mellitus (T1DM), subsequently diagnosed to have DIDMOAD syndrome. We also highlight atypical presentations and early pointers to the disease.

A 9-year-old girl was diagnosed to have T1DM 2 years back and was on insulin therapy. She presented with decreased visual acuity and polyuria (urine output 4 L/day) that persisted despite good glycemic control. On evaluation, urine osmolality was 158 mosmol/L and serum osmolality was 302 mosmol /L. Urine osmolality increased to 280 mOsm/L with intravenous vasopressin suggestive of central DI. Magnetic resonance imaging (MRI) brain revealed an absent pituitary bright spot. Detailed evaluation revealed hydroureteronephrosis, neurogenic bladder, bilateral optic atrophy and bilateral moderate sensorineural hearing loss. Direct sequencing of *WFS1* gene by Sanger method, revealed a novel homozygous variant frame shift mutation c.2486_2489dupTGGA (p.Glu830Asp*111) in exon 8. She was managed with oral desmopressin tablets, clean intermittent urinary catherization and continued on insulin therapy.

Five patients (3 males) with WFS type 1 were identified with the mean (SD) age of 11 (2) years. There was no family history or consanguinity in the parents. All patients had DM and OA. DI was present in 4 patients and hearing impairment and urological abnormalities in 3 patients each. All cases had normal stature except one, and all were pre-pubertal except case 3. Case 3 presented at 13 years with DM since 4 years, polyuria (despite adequate glycemia) and visual problems. Eye examination revealed optic atrophy and glaucoma. She was diagnosed to have central DI and started on desmopressin. On follow-up, she had delayed puberty (absent menarche till 16 years with SMR stage 3). The baseline gonadotropin levels were LH 1.09 mIU/mL and FSH 6.37 mIU/mL, and levels post-GnRH stimulation LH 20.6 mIU/mL and FSH 28.6 mIU/mL.

Thyroid function tests were done in all children and were found to be within normal limits. Case 3 reported compound heterozygous missense/frameshift mutation in exons 4/8 c.397G>A/c.1234_1237delGTCT (p.A133T/p.V412Sfs29). Case 2 revealed a homozygous deletion Exon 8 c.1525_1539 del15 (p.V509_Y513del) on genetic analysis by Sanger method and Case 4 revealed a novel homozygous variant missense mutation Exon 8 c.1372G>A (p.A458T). No mutation was identified in Case 5.

WFS is a rare neuro-degenerative autosomal recessive disease that was first described in 1938 [2]. Its prevalence was estimated to be 1 in 68,000 to 1 in 770,000 [2,3]. Apart from these common manifestations, screening for urological and psychological abnormalities, and endocrine disorders is paramount, as they often remain unnoticed, adding to disease morbidity. The minimum diagnostic criteria of WFS are the coincidence of early-onset DM and OA [2]. There is no effective treatment for this neurodegenerative disease with reports suggesting a median life expectancy of 30 years [2]. Death usually occurs from respiratory failure as a result of brain stem atrophy.

DM is usually the first manifestation of the disease. A multicentric study conducted by Rohayem, et al. [4] described notable differences between the diabetes of WFS and TIDM including earlier median age of onset of diabetes, less incidence of diabetic ketoacidosis at onset, a much lower insulin requirement, rare micro-vascular complications, and absence of autoantibodies in the former. The mean (SD) age of diagnosis of DM in our study was 8.2 years (2) with none of patients having diabetic ketoacidosis at presentation.

Diabetes insipidus appears at an average age of 14 years and affects approximately 70% of patients [3]. About 80% of the patients in our study had DI, which is consistent with the literature. The diagnosis is often delayed as polyuria and polydipsia are overlapping symptoms of both DI and DM.

Patients with WFS demonstrate progressive optic atrophy that usually occurs after diagnosis of DM. Other ophthalmological findings reported are colour vision deficits, cataract and pigmentary retinopathy [5]. All patients in our case series had optic atrophy, whereas glaucoma and cataract were present in two and one patient, respectively.

Bladder dysfunction in children and young adults with WFS is common and easily missed (only 30% symptomatic), and can be initial presenting feature as was seen with two of our cases. Structural and functional urinary tract abnormalities are commonly seen including atonic bladder, bladder-sphincter dyssynergia, hydro-ureteronephrosis, and recurrent urinary tract infections [6].

Patients with DIDMOAD have been reported to have growth failure due to defects in hypothalamic pituitary function [6] and hypogonadism, therefore follow-up of these patients is essential. Neurological complications generally appear in later life at a median age of 30 yrs (range 5-44yrs) and include truncal ataxia, loss of gag reflex, myoclonus, epilepsy, peripheral neuropathy and central apnea [1]. Psychiatric manifestations including depression, psychosis and aggression are also common and should be screened for.

In conclusion, our case series highlights a lack of awareness among physicians about this entity, culminating into under and delayed diagnosis of this disorder. There is a need to have a high index of suspicion for the diagnosis of DIDMOAD syndrome in patients with T1DM presenting with other systemic involvement. Patients with DIDMOAD should be screened for other associated problems and require multi-disciplinary care [6]. Since this is a neurodegenerative disorder with poor prognosis, it is prudent to provide appropriate genetic counselling and offer prenatal diagnosis for prevention in future pregnancies.

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Rare Inherited Hypomagnesemias - An Endocrine Case Series

Hypomagnesemia is a common dyselectrolytemia with serious manifestations. The purpose of the current series is to highlight the importance of systematically working up inherited hypomagnesemias. We describe five patients from three families with genetically proven hypomagnesemias. First family had twin sisters with Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) and second (two siblings) and third family had Hypomagnesemia with secondary hypocalcemia (HSH) both of which are rare inherited hypomagnesemias. Diagnosis was made only during systematic workup of hypomagnesemia many years after initial presentation.

Patients 1and 2: These were 22 year old twins incidentally discovered to have mild elevation of serum creatinine with bilateral medullary nephrocalcinosis at the ages of 15 and 18 and were being managed conservatively by nephrologist. They were referred to endocrinology for evaluation of high parathyroid hormone levels (PTH) (711.5 pg/mL and 589 pg/mL). Both were physically well on examination with a normal blood pressure and sterile pyuria. Evaluation revealed vitamin D deficiency (5.56 ng/mL and <3 ng/mL) secondary hyperparathyroidism, hypercalciuria and hypomagnesemia (1.5 mg/dL in both). Fractional excretion of magnesium (FEMg) was elevated in both (10.2% and 14.9%) indicating renal magnesium (Mg) wasting. FHHNC was suspected in view of mild renal failure, hypomagnesemia and nephrocalcinosis. Genetic testing revealed a novel heterozygous pathogenic mutation c.313G>A (p.D105N) in CLDN16 exon 1 in both the sisters. They were started on oral magnesium supplements and cholecalciferol which normalised their Mg and PTH values.

Patients 3,4 and 5: Patient 3 was a six year male child with refractory seizures since one month of age treated with multiple antiepileptic drugs (AEDs). Neuroimaging was normal. Baby was referred to endocrinology at four years of age in view of hypocalcemia. The child's elder female sibling (patient 4) had intractable seizures since one month of age and died at six months of age from status epilepticus. Her reports showed hypocalcemia and hypomagnesemia but treatment details were unavailable. Endocrine workup of patient 3 also showed hypomagnesemia (1 mg/dL), hypocalcemia (5.4 mg/dL) and normal PTH levels. His urine calcium creatinine ratio was 0.02, FEMg was <2% with no nephrocalcinosis. Clinical diagnosis of HSH was suspected. Genetic analysis revealed a novel homozygous mutation of TRPM 6 in intron 18 (c.2392 -3 T>G) with parents heterozygous for the same mutation confirming the diagnosis of HSH. He was started on high dose magnesium supplements with which hypocalcemia resolved and child has remained seizure free off AEDs. Diagnosis of HSH was made retrospectively in patient 4. Patient 5 was a one year old female child with recurrent seizures from five months of age and mild developmental delay. Workup revealed hypocalcemia (5.93 mg/dL) and hypomagnesemia (0.8 mg/dl). Initial FEMg was low (1.03%) when the serum Mg was low. After intravenous Mg loading, FEMg increased clinically confirming the diagnosis of HSH. Child is seizure free on magnesium supplements. Genetic testing reports are awaited.

FHHNC, first described as Michelis-Castrillo syndrome occurs due to mutations in the CLDN16 (chromosome 3) and CLDN19 (chromosome 1) genes, encoding claudin-16 and 19, respectively [1]. Claudins are paracellular proteins in the thick ascending limb (TAL) of Loop of Henle involved in calcium and magnesium reabsorption. Claudin-19 mutations are associated with macular coloboma, pigmentary retinitis and nystagmus. Only 100 patients with *CLDN16* mutations and 70 patients with CLDN19 mutations have been described till date in literature [2]. FHHNC typically presents in infancy with recurrent urinary tract infections, polyuria, convulsions and failure to thrive. Sterile pyuria, hypercalciuria, hypo-magnesemia and nephrocalcinosis are classical, with occasional incomplete distal renal tubular acidosis and hypocitraturia. Amelogenesis imperfecta has also been reported [3]. Approximately 50 % of FHHNC patients develop progressive renal failure leading to end stage renal disease (ESRD) in the second decade.

The distinguishing features of FHHNC are its progression to ESRD and uncommon occurrence of acute hypomagnesemia. Current treatment options include magnesium supplemen-tation, hydrochlorthiazide and prostaglandin antagonist/(indomethacin). Renal transplantation is the definitive treat-ment [4]. Discovery of claudin-14 as an inhibitor of claudin-16/19 complex has made it an upcoming therapeutic target [5].

HSH or Primary intestinal hypomagnesemia due to TRPM6 mutations has been reported only in 50 cases so far. TRPM6 gene (chromosome 9q22) encodes a magnesium channel in the distal small intestine and renal distal convoluted tubule. This is the most severe form of inherited hypo-magnesemia, autosomal recessive modulated by x linked gene [6]. Hypocalcemia can be due to PTH resistance, impaired PTH release or impaired vitamin D synthesis, but the exact mechanism is unknown. Infants present with recurrent seizures and tetany. Adults present with hypertension, arrhythmias or osteomalacia and keratoconus in third decade. The diagnostic feature is a low FEMg at baseline which increases when serum Mg is normalized by intravenous Mg supplementation indicating impaired intestinal Mg absorption as well as renal Mg wasting. High doses of elemental magnesium (0.7-3.5 mmol/kg/day) ensures normalization of calcium even if Mg remains at low normal levels. Hence, whenever a child presents with refractory seizures and hypocalcemia, Mg should be checked and if low, HSH should be suspected. HSH responds poorly to high dose AEDs and calcium leading to poor neurodevelopmental outcome and even death as in patient 4 but completely recovers with magnesium supplementation as exemplified in patients 3 and 5. Till date, only one case each of genetically proven FHHNC and HSH have been reported from India. Inherited hypomagnesemias though rare should be suspected in appropriate clinical settings which will help us to prevent major morbidity and mortality. Workup of refractory seizures should always include measurement of serum calcium, magnesium and its fractional excretion.

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Acute Necrotising Encephalopathy of Childhood Secondary to Rotaviral Diarrhoea

A one-year-old male child presented with complaints of fever, vomiting and loose stools for 4 days. After 6 days, symptoms subsided but child developed rapid worsening sensorium and hepatomegaly. Investigations revealed marked elevation in serum transaminases (aspartate aminotransferase (AST) 10491 IU/L, alanine aminotransferase (ALT) (8990 IU/L), serum albumin was 2.4 gm/dL, prothrombin time, 32.5 seconds (International normalized ratio (INR) was 2.77, which improved to 1.53 after vitamin K supplements. Serum ammonia was initially 136.7 µg/dL which improved to 23 µg/dL on treatment. Cerebrospinal fluid analysis (CSF) was normal. Dengue NS1, IgM and IgG, HBsAg, hepatitis C IgG, hepatitis A IgM, hepatitis E IgM and ELISA for HIV were negative. Stool rotavirus antigen was positive.

Magnetic resonance imaging (MRI) of brain showed hyperintensities in the bilateral caudate nuclei, putamen, globus pallidus and restricted diffusion in the bilateral basal ganglia suggestive of acute necrotizing encephalopathy. Child was treated with intravenous methyl prednisolone (30 mg/kg/day) for 3 days, followed by oral prednisolone at 2 mg/kg/day for 15 days, which was gradual tapered over next one month. On day 6 of hospitalisation, the child's sensorium improved and he was discharged with feeding tube in situ, with residual neurological deficit, and AST of 81 IU/L and ALT of 1250 IU/L. On follow-up after 45 days of illness, his liver function tests have normalized, he can feed without the feeding tube, can speak monosyllables and can recognize parents.

Acute necrotizing encephalopathy is a para-infectious disease triggered by viral infections, most commonly by influenza and HHV 6 [1-3]. The most likely hypothesis for the pathogenesis of ANE is the exaggerated inflammatory response to viral infection leading to liver dysfunction, acute renal failure, shock, and disseminated intravascular coagulation. In nervous system, the permeability of vessels is altered without vessel wall disruption [3].

Our patient had a history of viral gastroenteritis and the stool rotavirus antigen was positive. Neurological manifestations associated with rotavirus have been described [4]. Thus, we consider rota virus as the possible etiology for ANE. Definitive treatment guidelines for ANE have not been formulated but antiviral therapy, immunomodulatory treatment, antithrombin III, therapeutic hypothermia and cyclosporin A have been variably used [1,3]. ANE is associated with a high mortality and less than 10% of patients recover completely [3]. In conclusion, a high index of suspicion for ANE is needed in a previously healthy child with sudden onset neurological symptoms following acute febrile illness.

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The Neonatal Resuscitation Protocol: Keep It Simple?

Neonatal intensive care practice has its moments I would say; several actually. Sending a micropreemie home, watching a meconium aspiration pneumonia improve on high frequency ventilation and nitric oxide, managing to insert a life-saving central line catheter into a fine thread like vein, cherishing the normal neurodevelopment of a critically ill infant; all of these and more make the effort worth it. Why then, did the neonatologist in me decide to put myself at risk of criticism with this manuscript which could ripple some still waters? Albeit I am no virtuoso in the field of medical research, having faced the maelstrom of intensive care on my feet for over 10 years, I wish to comment on a guideline that makes a difference to every day practice. The neonatal resuscitation protocol (NRP) for the term newborn, can be described as "daily bread" to the genus of intensivists called neonatologists and has undergone several modifications over the last decades. Thyself followed, with great fervour, the 'reforms' made to the protocol [1]. Over the ensuing paragraphs I intend to raise my reservations on the tipping balance in NRP, between the quest for evidence based practices and pragmatism.

Those who work with older children and adults know that their patients crash mainly due to cardiac reasons. Basic life support training hence focusses on pushing hard and fast to get the circulation going [2]. On the other hand, babies who do not seem fine at birth are typically so because of prior hypoxemia and all the problems that result: hence, the focus on 'breathing'. Either stimulate him (forgive the literal gender bias) to cry, or drive air in by positive pressure ventilation, and most of the other problems (bradycardia, poor tone) sort themselves out. There is a small proportion who need chest compressions and drugs, though [3]. Experiments on animals, and results from various clinical studies, apparently guided changes in the protocol and aimed at improving outcomes, which is indubitably the correct way forward.

Did we outbid practicality in this quagmire of trying to add more and more boxes and branches to the resuscitation algorithm? Ab initio, everything seemed undeniably robust. Certain vital information was essential at birth (the first 'box' in the algorithm), and all it took to decide if the baby was fine was that he should cry with gusto and look all flexed and pink within minutes. But the contents of these so-called boxes kept changing, with its members moving in and out with every update. Even if you manage to let that pass, some of the additions that came in later further along the algorithm does want to make you sit up and roll your eyes. It sounded reasonable to attach a pulse oximeter probe for those who needed more supports; after all, we swear by *primum non-nocere* and oxygen does do harm [4]. But sticking ECG leads as time ticks on? And then staring at the monitor for precious seconds to get readings and act: now that is a tough one to comprehend in the chaos. Specifically when the evidence is tenuous at best; as slippery as the baby in fact [5]. Whatever happened to years of training listening to the lub-dub through the instrument we wield as the mark of a doctor?

Let that go by too. What seemed completely overboard and actually made me gape incredulously was this. Suppose the little fellow needs chest compressions too (remember someone is already ventilating him from the head end by then); the 'compressor' who was on one side of the baby needs to move to the head end and nudge the 'ventilator' to swap positions. The picture that comes to mind is that of an entangled crochet of the operators' forearms and hands. Why not continue the two-thumb chest compression from one side, allowing the airway person to continue his good work from the head end (especially considering the stability of the more important airway)? Seemingly to make way for a third person to insert the umbilical vein canula, if required. What happened to the other side of the baby, usually the left? Try telling me that clinical examination and procedures need to be done from the right side of the patient (one of the first 'rules' drilled into a medical student). The foundation for this custom is quite simply convention [6]. A right hander may do a better job while examining asymmetric organs like the liver, spleen or heart; but the umbilical stump? Try it on a mannequin, makes no difference at all- right or not right!

I could go on and on. Adding to the angst is the concern that a student/ trainee is reprimanded; or worse-still, failed in the objective structured clinical examination stations, for not strictly adhering to the rule-book. Not to mention the ever looming medico-legal issues that can be pursued by those who go to court for 'errors' during resuscitation.

At the end of the road, all we need to do most of the time, is to reverse the hypoxemia and hypercarbia by effectively ventilating the lung, and the heart and brain follow suit. But no, we like to complicate everything. I am reminded of the historical Rube Goldberg machine!

On a more serious note, if we set aside a miniscule fortunate fraction of the population, our country's less privileged interiors are still grappling with bare minimum availability of equipment and trained personnel [7,8]. With great efforts, the neonatal mortality has dropped from 38 to 23.5 per 1000 live births [9]. But we have miles to go. A close look at the vast differences in statistics within the country seems to indicate the need for very specific regional and local microplanning. In such conditions, we need to earnestly contemplate the practical applicability of the ever evolving NRP; and consider local, regional logistics and readiness before blanket recommendations are made. An additional section in the NRP guidelines on adoption of new guidelines at various levels of healthcare may be added to address similar issues.

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Three vs Four Dose Schedule Hepatitis-B Vaccine in HIV-infected Children

Jain, *et al*. with respect to the recent publication by [1] on the above topic, we seek the following clarifications:

Abstract mentions trial participants being fifty (25 per group) HIV-infected children aged 18 months - 12 years receiving ART for at least 6 months who had not received any prior dose of HBV vaccine, and were anti-HBs negative [1]. While in methods section it is mentioned as participants being seronegative for Hepatitis B virus (HBs antigen negative). If participants were anti-HBs antibody titre negative or HBsAg antigen negative? Or both antigen and antibody negative? Please clarify this confusion.

Regarding immunization status of participants, methods section mentions that immunization status was ascertained on the basis of previous immunization records [1]. Hepatitis B vaccination in immunization schedule of Delhi was introduced more than a decade ago [2]. So either participants were completely unvaccinated for all vaccines or vaccinated for all vaccines along with hepatitis B, depending on at what age they voluntarily stopped getting vaccines intentionally. So, no immunization record with no history of immunization too would have been a better proxy for unvaccinated subjects. How participants were left out for hepatitis B vaccine only? A previous randomized trial on similar topic [3] had subjects that were older, as routine hepatitis B vaccination had started just 1-2 years prior to the study.

Due to the convenience sampling, it is still unclear if double strength (20 µg) 4-dose schedule (0, 1, 2 and 6 months) is equally efficacious or superior to 3-dose schedule (0, 1 and 6 months), as the study was not powered to detect a difference unanswered thereby leaving this important question.

Baseline characteristics table shows mean age of groups I and II being 7 and 11 years, respectively [1]. It seems to differ significantly despite SNOSE technique and block randomization. Moreover, CONSORT flow chart shows 70 participants being eligible. While during enrollment 40 (on summing up) were excluded.

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AUTHORS REPLY

We thank the readers for their interest in our work [1] and provide the following clarifications: Regarding the inclusion criteria of participants, we wish to clarify that HIV-infected children aged 18 months - 12 years who had been receiving ART for at least 6 months and who had not received any prior dose of HBV vaccine were eligible provided they were seronegative (HBs antigen negative). Immunization status for hepatitis B was assessed by studying the immunization cards of the child as well as absence of anti-HBs antibodies. We had noticed that the immunization records of some of these children were incomplete due to reasons like relocation/ migration, or where the parents had succumbed to HIV. Hence, relying solely on immunization records and history, did not seem a robust method.

Although, Hepatitis B vaccination had been introduced in several Indian states almost a decade ago, the coverage of hepatitis B vaccine was reported low with huge gaps in coverage of DPT3 and HBV3 persisting [2]. A survey from India [3], reported that in 2015-16, 45% of the children aged 12-59months were not fully vaccinated against hepatitis B, and 20% children had not received even a single dose of hepatitis B vaccine. Some of the participants in our study had been born in remote rural areas and had later migrated to Delhi, and therefore had not received hepatitis B vaccine. Some of these children had also not received other vaccines; the missing vaccination doses were administered by us during their visits to the anti-retroviral clinic.

The disparity in ages of participants in both groups despite block randomization may have been due to the small sample size and because we did not perform stratified randomization [4]. We excluded 20 children out of 70 eligible children. The CONSORT diagram depicts that 20 children were excluded and also elucidates the reasons for exclusion [1].

We agree that the research question addressed remains unanswered. Finding even 50 children who had never received any dose of hepatitis B vaccine was very challenging for us, and hence a convenience sampling was done. This question may be answered by pooling similar data from other studies and performing a meta-analysis.

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Intravenous Acetaminophen vs Intravenous Diclofenac in the Management of Painful Crisis in Sickle Cell Disease

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We appreciate Panda, et al. [1] for their work on efficacy of intravenous (IV) acetaminophen and diclofenac for the management of pain in patients with Sickle cell disease (SCD) vaso-occlusive crisis (VOC). However, we would like to comment on few aspects of this article.

i) In the Introduction section, authors have mentioned "*IV diclofenac is the current standard of care for management of skeletal VOCs in SCD*" [1] but the guidelines suggest the management of acute pain in sickle cell VOC is based on the severity of pain. In patients with mild to moderate pain, non-steroidal anti-inflammatory drugs (NSAIDS) can be used, unless contraindicated, whereas opioids are recommended as first line drugs in patients with severe pain [2].

ii) Authors have stated that oral NSAIDs are associated with gastric side effects [1]. The primary mechanism of gastritis by NSAIDs is by inhibition of prostaglandin production which is caused by both oral and parenteral NSAIDs [3]. Albeit less common, the risk of gastritis with parenteral NSAIDs cannot be ruled out.

iii) IV acetaminophen dose ranges from 10-15 mg/kg/dose and its effect lasts for 4-6 hours [4]. We fail to understand why paracetamol was given at 10 mg/kg/dose at 8-hour intervals.

iv) In the methodology section, authors have mentioned that patients who did not improve after home-based care and were symptomatic within 24 hours were included in this study. Many of these patients would have taken oral NSAIDs, particularly diclofenac, before reaching hospital. These patients should have been excluded from the study, as this could have an impact on the overall response rate.

v) In the result section, we found only 5 (4.91%) patients required add on therapy out of 102 patients included in this study, which signifies a remarkable response to both these drugs in acute crisis. Mean (SD) number doses required for complete relief of pain were 6 (4) and 8 (4) in the acetaminophen and diclofenac group, respectively. In our opinion patients who had more than 50% reduction in pain within 24 hours could have been switched over to oral drugs rather than prolonged parenteral therapy.

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AUTHORS REPLY

i) We agree that opioids are still the standard of care for severe pain in SCD skeletal VOC, but IV diclofenac is the current standard of care for management of skeletal VOCs among HbSS children in our center, as opiates are not freely and continuously available, there is a lack of manpower to closely monitor respiratory depression in a high volume center, severe constipation with regular usage of opiates, more likelihood to develop opioid dependence in patients with severe and frequent VOCs, and gastric side effects with regular usage of oral NSAIDs. Moreover, we had observed that most patients coming to us with mild to moderate pain had already taken oral NSAIDs without relief.

Thus, due to non-availability of opioids, observed non-response to oral NSAIDs, and possibility of nephropathy with chronic diclofenac use, we planned this study.

ii) We agree with this statement.

iii) The dose range of IV paracetamol is 10-15 mg/kg/dose with duration varies from 4 to 8 hour, depending upon the situation. We enrolled only those patients who responded to 8-hly regimen, for ease of analysis.

iv) We included only those patients who had not received any medications, and home- based care means only taking sufficient fluid and restricted outdoor activities to prevent dehydration.

v) We agree that patients who had more than 50% reduction in

pain within 24hrs could have been switched over to oral drugs therapy and the same was also done by us. However, as our study end point was achieved, we have not mentioned these in our manuscript.

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Feeding Schedule in Preterm Infants: Two hourly versus Three Hourly

We read with interest the recently published randomized controlled trial by Yadav, et al. [1] comparing two-hourly and three-hourly feeding schedule in very-low-birth-weight neonates. We seek the following clarifications:

i) It is not clear whether the neonates were randomized at birth, at the time of introduction of feeds or at a specific time point within the first 96 hours. This is important, the time of randomization has a direct bearing on the primary outcome.

ii) The authors mention that the subgroup analysis was as per birthweight (1000-1250 grams vs >1250 grams), however, the same is not reported here. This subgroup analysis is vital and will help in increasing the generalizability in babies <1250 grams.

iii) In this trial, 40% of the enrolled neonates were small for gestational age (SGA) who are at higher risk for feed intolerance, hypoglycemia, and necrotizing enterocolitis (NEC) [2]. Therefore, it is desirable to have a subgroup analysis for SGA neonates for the above-said outcomes.

iv) What was the rationale for excluding infants with the absent or reversed end-diastolic flow? A recent large body of evidence did not show any interaction between antenatal absent or reversed end-diastolic umbilical flow and feeding advancement [3].

v) One of the major rationales of doing this trial was that three hourly feeding intervals might reduce nursing time in a resource-constrained setting. The previous study has shown that three hourly feedings are associated with shorter nursing time per infant [4]. It is desirable to have this data.

vi) Probiotic use can have a direct impact on mortality and NEC rates and may act as a confounder. Therefore, it is desirable to compare the probiotic use among two groups.

vii) Though the authors have presented time to full enteral feeds, many preterm neonates (<1250 grams) must be on tube feeds at the time of enrolment. It will be interesting to know whether there was any difference among the two groups in the time to reach full oral feeds (spoon/paladi/cup) and the duration of the transition in neonates who were on tube feeds at enrolment.

Recently a group of researchers advocated that the clinical trials should choose uniform outcome measures and report all clinically relevant outcomes for uniformity [5]. For trials related to feeding a set of important clinical outcomes shall also include weight gain (g/kg/d), time to regain birth weight, length of hospital stays, duration of parenteral nutrition, sepsis rates, along with other vital outcomes like retinopathy of prematurity and bronchopulmonary dysplasia. The authors should report this data to improve the generalizability of the study.

We sincerely believe that the clarification of the above points shall be immensely helpful for the clinicians and researchers.

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AUTHORS REPLY

We appreciate the authors' interest in our study [1], and provide the clarifications:

i) The neonates were approached for randomization within first 96 hours and were enrolled as soon as the participants were deemed fit for inclusion. However, we did not record exact time of randomization or initiation of feeding.

ii) We agree with the point about subgroup analysis based upon weight and small for gestational age status. Detailed analysis shall be published later. There was no difference in time to reach full enteral feed, hypoglycemia, feed intolerance or necrotizing enterocolitis (NEC) among small for gestational age (SGA) neonates too (**Table I**). This finding is reassuring and indicates the applicability of

Table I Comparison of Primary and Secondary Outcomes as per Appropriateness for Gestational Age

Outcomes	*Two-hourly group (n=110)*	*Three-hourly group (n=99)*
Appropriate for gestational age		
Time to reach full enteral feeds (n=100)[a]	5.27 (1.73)	4.90 (1.17)
Hypoglycemia	4 (3.64%)	3 (2.97%)
Episodes of feed intolerance	8 (7.27%)	5 (5.1)
Necrotising enterocolitis	0	2 (1.98%)
Small for gestational age		
Time to reach full enteral feeds (days)*	5 (1.49)	5.36 (2.09)
Hypoglycaemia	2 (3.08)	4 (5.41%)
Episodes of feed intolerance	5 (7.69%)	7 (9.46%)
Necrotising enterocolitis	4 (6.15%)	3 (4.05%)

Values in no. (%) except [a]mean (SD). All P values >0.05.

trial to growth restricted neonates which are considered at higher risk for adverse outcomes. The SGA neonates were overall at significantly higher risk for NEC (7 vs 2; *P*-0.016) as compared to appropriate for gestational age, irrespective of feeding schedule.

iii) We agree with the point raised over excluding neonates with absent or reversed end diastolic flow (A/REDF). However, at the time of commencement of the study (2017) our unit policy was withholding feeds for first 24 hours and thereafter slow advancement of feeds (10-20 mL/kg/day) in neonates with A/REDF [2]. For the index study we planned rapid advancement of feeds (30 ml/kg/day) for all enrolled infants and the team was worried over the rapid advancement of feeds in A/REDF population. Therefore, inclusion of neonates with A/REDF would have either led to deviation from the protocol. Also, as of now, three-hourly feeding is not a standard of care. Therefore, to ensure uniformity in study protocol and to ensure safety we excluded neonates with A/REDF. We are also aware of the Cochrane review published in 2017 (after commencement of our study) showing no evidence of increase in NEC with rapid advancement of feed in these neonates [3].

iv) We agree that it is an important outcome. However, we did not objectively record this data.

v) Bone of the neonates in the study received probiotics.

vi) Due to high volume of admissions and rapid turnover we did not record time to reach full oral feeds and the duration of the transition in neonates who were on tube feeds at enrolment. However, as per our policy the spoon feeds are started at 31 weeks of postmenstrual age.

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Artificial Intelligence in Medical Education

Artificial Intelligence (AI) is bringing a great transformation in all spheres of life including healthcare sector. Recent work has proved that AI techniques have a great potential in making healthcare facilities affordable and easily accessible [1]. AI promises early diagnosis of diseases, improved patient care and facilitate continuous monitoring of patients. However, for an optimized use of AI for healthcare, doctors and AI experts need to collaborate. Thus, it is desirable that medical graduates have a good understanding of data science and AI techniques, they are going to need to handle it in the near future.

Researchers have developed powerful high-performance AI tools in healthcare to predict the occurrence of many chronic diseases like cancers, diabetes, etc. [3]. Many AI tools have already been approved by regulatory authorities to diagnose diseases, and are being used in primary healthcare centres of rural areas in developed countries like USA [3]. As the healthcare policy-makers are looking forward to amalgamating AI in healthcare sector, it is high time that medical education curriculum be updated to include formal education of emerging medical professionals in this technology. Introduction of AI in medical education curriculum has previously also been suggested [4]. Curriculum should be focussed on AI literacy rather than expertise. AI researchers/data scientists may act as resource persons to conduct faculty development programs in AI for medical faculty. The subject must be taught making sure that complex mathematics is avoided, and the concepts are explained in an easy way. For the medical students, emphasis should be laid on population health and evidence-based medicine. Clinicians should have a formal training of using AI tools to spot anomalies, forecast patterns from medical data and make decisions. Medical students may be provided with an opportunity to see and observe simple concepts of data mining working with small data science projects to enable them to use AI techniques to get meaningful information from data.

There is a pressing need to build an ecosystem where AI experts, data scientists and medical practitioners collaborate to ensure optimal utilization of AI in healthcare sector. For this to happen, it is desirable to orient medical student of today to AI to enable him to use the same tomorrow.

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Cerebral Abscess: A Delayed Complication of Electrical Burns

A 6-year-old boy suffered electrical injury when his head accidentally came in contact with a loose electrical wire of a room cooler. The child lost consciousness transiently and presented with burns on the scalp to a local practitioner. The entry and exit wounds were noticed in the parieto-temporal areas on the right and left sides of the scalp, respectively. Neuroimaging revealed tiny hemorrhagic contusions in the right frontal and parietal areas. Child received oral antibiotics and daily dressing. Two months later, the child presented to us with fever and left-sided focal seizures of one day duration, along with history of episodic irrelevant talking and shouting during the preceding two days. On examination, the child was conscious, with weakness in the left upper limb and left-sided supranuclear facial nerve palsy. The deep tendon reflexes were brisk with bilateral extensor plantar reflexes. The child did not have any signs suggestive of meningeal irritation and there was no papilledema. The laboratory investigations were unremarkable. Magnetic resonance imaging of the brain revealed an ill-defined lesion (38 × 27 × 36 mm) with peripheral blooming in the right frontoparietal lobe with significant perilesional edema, associated with peripheral enhancement of the lesion with associated patchy leptomeningeal enhancement. Focal calvarial thinning was seen in the left posterior high parietal region. A possibility of right cerebral abscess with associated cerebritis and meningitis was kept. The child was treated with intravenous ceftriaxone, vancomycin and metronidazole along with intravenous phenytoin and mannitol. The child had repeated uncontrolled seizures and died within 24 hours, before neurosurgical intervention could be done.

The presentation of electrical injuries in children can be unique as these may involve uncommon sites and the severity may be greater as the percentage of fat may be lesser as also the different surface area to volume ratios compared to adults [2]. Low voltage circuits seen in domestic settings are usually less damaging, however, alternating current is more injurious than direct current. Blood vessels and brain tissue, due to the high fat content, are more vulnerable to thermal effects of current [3]. Previously, electric burn of skull in association with cerebral contusion and intracranial infection was reported in a patient who had a successful outcome following timely surgical intervention [4]. Unfortunately, neurosurgical intervention could not be undertaken in this child due to delayed presentation in the hospital.

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The SARS-CoV-2 variants of concern

As the SARS-CoV-2 pandemic unfolds the new villains in the drama are 3 viral variants of concern (VOC) which have recently emerged. The B.1.1.7 was described in the UK. The B.1.351 surfaced in South Africa and P.1 was born in Brazil. What do we to know about these viral variants?

All the three variants have the N501Y mutation in the receptor binding site of the spike protein. The B.1.351 and P.1 also have two additional mutations which again affect the receptor binding domain. These mutations have resulted in increased binding to the ACE 2 receptor and subsequently higher infectivity. The British variant is 36-75% more contagious. The South African variant is 50% more transmissible. Early data from Brazil suggests that the P.1 is 2.5 times more contagious.

What about disease severity? Initial studies suggested that the UK variant, B.1.1.7 did not cause more severe disease. However, more recent data, suggests that the mortality is more in the new variant. The mortality hazard ratio in the new variant as compared to the old was 1.64 (95% CI 1.32-2.04). This translates into a death rate of 4.1/1000 cases for the new variant against 2.5/1000 in the old variant. In South Africa the mortality due to the new variants was 20% more than the previously reported death rates. It could also be due to the rapidly overburdened health care system.

The million dollar question which remains is whether vaccination or previous infection will protect against the new variants. Sera from individuals immunized with the Moderna vaccine showed that they efficiently neutralized the UK variant B.1.1.7. Its efficacy was slightly reduced for the South African strain but will probably be effective clinically. However, Moderna has announced that they will develop a new modified vaccine which will be effective against the new strains.

Serum from patients vaccinated with the Pfizer- BionTech vaccine also had a slightly lesser efficacy in neutralizing the new variants but the vaccine will probably remain clinically effective. Serum from patients vaccinated with the AstraZeneca vaccine failed to neutralize the new variants.

Initial data suggests that natural immunity due to previous infection with the old variant is partially effective in preventing infection with the new UK strain but hardly effective in preventing infection with the South African strain. More concrete data is awaited.
(*New SARS_CoV-2 variants- Clinical, public health, vaccine implications. NEJM 24 March 2021*)

mRNA vaccines- transforming medicine

In addition of their sucess against SARS-CoV-2, mRNA vaccines are also in the race to treat several non-infectious disorders.

It was known for decades that mice injected with mRNA could produce the proteins that the mRNA coded for. However, the mRNA itself produced a severe inflammatory response. Secondly the mRNA would get degraded very fast in the body which led to low levels of protein production.

The major breakthrough came from the work of a pair of scientists from the University of Pennsylvania - Katalin Kariko and Drew Weissman. They found that using synthetic nucleosides to produce the mRNA prevented the inflammatory response and also resulted in more protein production. The next milestone was the imaginative use of lipid nanoparticles to coat the mRNA. This prevented the rapid degradation of mRNA.

When the COVID 19 pandemic swept the world, we needed a safe vaccine which could be rapidly developed at low cost. mRNA vaccines ticked all the boxes.

Now, Ugur Sahin, CEO of BionTech and his group have published another remarkable piece of research. They developed a mRNA which delivers auto antigens of multiple sclerosis into the lymphoid dendritic cells. This activates a regulatory T cell which inhibits the inflammatory response against the targeted autoantigens. In mouse models of multiple sclerosis, this mRNA vaccine delayed the progression and reduced the severity of the disease. Most importantly unlike the regular therapy of multiple sclerosis it does not induce generalized immunosuppression.

So besides its potential role in emerging infectious diseases, mRNA vaccines are purported to be the next blockbuster in the therapeutic arsenal against chronic diseases like cancers, cystic fibrosis and heart disease.
(*Krienke et al. Science, 8 January 2021*)

Nightingale wards

Florence Nightingale pioneered the idea that the design of hospital wards had a critical impact on the well-being of patients. The so called Nightingale wards had large windows, which allowed fresh air and sunlight to flood the rooms. Steven Lockley who studies circadian rhythm and sleep in Harvard Medical School has discussed how right Nightingale was when she highlighted the role of natural light in human health and well-being.

Patients in rooms with good natural light and outdoor views have been shown to recover faster with fewer painkillers. Heart rates, blood pressure and mood is better in these patients. Rods and cones were initially considered to be the only cells involved in visual inputs. It is now well established that non visual photoreceptors in the retina modulate our circadian rhythm, mood, alertness and cognitive functions. A large study from Korea analyzed length of stay of patients admitted with a bed next to a window versus away from a window. Patients with beds next to a window had much shorter hospital stays.

The COVID-19 pandemic has further emphasized the need for sunlight and cross-ventilation. There are many factors we need to consider while treating patients, and hospital design is often neglected.
(What Florence Nightingale can teach us about architecture and health. Scientific American 18 March, 2021)

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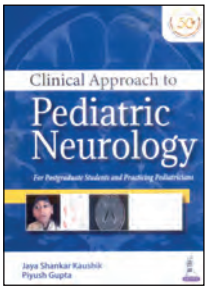
BOOK REVIEWS



Clinical Approach to Pediatric Neurology
JAYA SHANKAR KAUSHIK AND PIYUSH GUPTA
Jaypee Brothers Medical Publishers (P) Ltd., New Delhi.
Pages: 452; *Price*: Rs. 1495/-

This book completely justifies its title. It provides ample reading material to anyone interested in the field of clinical pediatric neurology. The authors use a pragmatic approach to describe day-to-day problems faced by a practicing pediatrician. Those pursuing specialized training in the field of pediatric neurology also stand to benefit from this book. The book is arranged into four sections with a total of 32 chapters. The section-3, "Resident's Corner," is unique and targets the exam-going residents. It discusses nine cases of particular interest to those who are appearing for exams. It provides all relevant information needed to prepare present cases in the exam. There is liberal use of tables, illustrations, images, and clinical photographs to enhance the reading experience. All in all, the book is a concise and pragmatic text that will be of help to practicing pediatricians and students alike.

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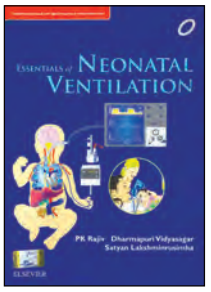

Essentials of Neonatal Ventilation (2019)
PK RAJIV, DHARAMPURI VIDYASAGAR AND SATYAN LAKSHMINRUSIMHA
Elsevier RELX India Pvt Ltd., Gurgaon, Haryana,
Pages 878; *Price*: *Rs*. 2570/-

Rapid evolutionary changes are occurring in neonatal ventilation and provision of respiratory support is a frequent reason for admission to the Neonatal intensive care unit (NICU). Hence, neonatal ventilation is a must know area for neonatal intensivists and students of neonatology. A thorough understanding of development of respiratory system, pathophysiology of respiratory diseases, and the equipment for respiratory support is central to quality ventilatory care of the newborn. This book is an effort towards that end.

The book includes 40 chapters under 8 sections written by more than ninety international experts mostly from the USA, UK, UAE and Europe. It covers lung development, applied physiology, ventilatory strategies for critical respiratory diseases, targeted echocardiography, near-infrared spectroscopy, pulmonary graphics, and modes of ventilation including noninvasive, high frequency and neurally adjusted ventilatory assist. Multicolored illustrations have been used where required, particularly for lung development, pathophysiology and pulmonary graphics.

The book will be useful for residents, postgraduates, pediatricians and neonatologists working in the NICU.

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