Original Research Article

Thrombotic risk in children with idiopathic nephrotic syndrome in Abidjan

ABSTRACT:

Aims: The objective of this study was to evaluate biologically, the thrombotic risk in children with idiopathic nephrotic syndrome (INS) in Abidjan.

Study location and period: This study was conducted in 56 children with INS, aged 24 to 144 months and followed up at the pediatric nephrology unit of the University Hospital of Yopougon. It took place from January 2021 to April 2022. Blood samples were taken in that unit for each child. Blood counts and albumin levels were performed at the laboratory of biology and medical research of the National Institute of Public Health (INSP) in Adjamé. Fibrinogen, factors V, VII, VIII, IX, antithrombin III (ATIII), protein C (PC), protein S (PS) and D-dimers were measured at Laboratory Longchamp of Plateau.

Methodology: In each child, blood samples were collected by venipuncture on dry tubes, with sodium citrate and EDTA. Blood counts were performed on Sysmex XT 2000i, albumin levels on Cobas C311 and haemostasis parameters on Sysmex CS1600.

Results: there was a predominance in male children. Children aged 24-59 months were in the majority (69.6%). Hypoalbuminemia < 30 g/L was found in 35 (62.5%) children. The most common haemostasis abnormalities were increased D-Dimer in 35 (62.5%) children, hyperfibrinemia in 34(60.7%) children, increased CP in 31 (55.3%) children, thrombocytosis in 29 (51.8%) children. ATIII and PS deficiencies were found in 16 (28.6%) and 11 (19.6%) children respectively. Thrombosis risk was present in 36 (64.3%) children. A significant association was found between albuminemia < 20 g/L and ATIII < 70%.

Conclusion: There is a thrombotic risk in children with INS, which may be increased by hypoalbuminemia < 20 g/L.

Key words: Idiopathic nephrotic syndrome, Thrombosis, Child, Abidjan.

1. INTRODUCTION

Idiopathic nephrotic syndrome (INS) is a renal disorder resulting from damage to the glomerular capillaries, which become more permeable to protein [1]. In children, it is characterized by massive proteinuria above 50 mg/kg per day, a hypoalbuminemia below 30 g/l and/or concomitant edema [2]. Proteins escape into the urine, resulting in proteinuria, which reduces its concentration in plasma, leading to hypoprotidemia. This hypoprotidemia is the cause of most of the clinical consequences of INS, namely infections, dyslipidemia and thrombosis due to loss of anti-coagulant proteins and overproduction of pro-coagulant factors [3, 4]. No study was carried out in Côte d'Ivoire on the risk of thrombosis in children with INS, hence the aim of this study, which was to evaluate the thrombotic risk of children with INS in Abidjan from a biological point of view.

2. PATIENTS AND METHODS

2.1 Study population

A descriptive and analytical cross-sectional study was conducted from January 2021 to April 2022. This study was carried out in children aged between 24 and 144 months, with INS and followed up at the unit of pediatric nephrology of the University Hospital of Yopougon. Any child with non-compliant samples was excluded from the study, and any child with a urinary tract infection was excluded from the study. Over the study period, 56 children with INS were collected. The characteristics of study population were summarized in Table 1.

Table I: Distribution of children by age and sex

Parameter	Frequency	Percentage
Age groups (month)		

[1-59]	39	69.6
[60-119]	15	26.8
[120-180]	2	3.6
Total	**56**	**100**
Sexe		
Male	35	62.5
Feminine	21	37.5
Total	**56**	**100**

2.2 Methods

Patients were recruited and samples were taken at the unit of pediatric nephrology of the University Hospital of Yopougon. Nephrotic syndrome (NS) was considered idiopathic in all children aged between 24 and 144 months at the time of diagnosis, and whose etiological work-up (HIV, malaria serology, hepatitis, bilharzia serology) was negative. Blood counts and albumin levels were performed at the laboratory of biology and medical research of the National Institute of Public Health (INSP) in Adjamé. Haemostasis parameters such as fibrinogen, factors V, VII, VIII and IX (FV, FVII, FVIII and FIX), antithrombin III (ATIII), protein C (PC), protein S (PS) and D-dimers were measured at the Laboratoire Longchamp of Plateau. Sociodemographic data were collected using a survey form. After enrolment, violet, red and blue tube samples were taken from children with INS, to obtain the biological specimens used for the various biological tests. Whole blood collected in EDTA tube was used to perform blood counts on Sysmex XT 2000i manufactured in Kobe (Japan), using two processes which are impedance and optics, to obtain the platelet count for each child. The sera, obtained after centrifugation at 1500 rpm for 5 minutes, were used to determine albumin levels using a colorimetric test on Roche Cobas C311 manufactured in Tokyo (Japan). The plasmas, obtained after double centrifugation of the citrated tubes at 3000 rpm for 15 minutes, were used for haemostasis tests on Sysmex CS1600 manufactured in Kobe (Japan). The tests involved fibrinogen, factors V, VII, VIII and IX, measured using the principle of the

chronometric method; ATIII and PC, whose assays are based on the principle of the colorimetric method. These assays made it possible to evaluate the activity of these different parameters. Finally, PS and D-Dimers were measured using an immunological test to obtain their concentration.

The normal values in children of the various biological parameters studied established in the literature allowed the interpretation of the results [5, 6, 7, 8, 9].

2.3 Data processing and analysis

Data were entered and analyzed using Excel 2013 software. The analytical results from the samples were used to determine the main statistical parameters, namely the means (m) and standard deviations (s) when the values of the distribution followed the normal distribution, and the median (Med) [1st quartile (Q1), 3rd quartile (Q3)] when the values of the distribution did not follow the normal distribution. Fisher's exact statistical test was used and considered significant if $P < .05$.

3. RESULTS

The mean values of the parameters studied were in favor of thrombocytosis, hypoalbuminemia, hyperfibrinemia, increased Protein C and high D-Dimers (Table II). The most common abnormalities were hypoalbuminemia <30 g/L and D-Dimers ≥ 500 ng/ml in 35 children (62.5%), hyperfibrinogenemia > 4 g/L in 34 children (60.7%), increased protein C in 31 children (55.3%), thrombocytosis in 29 (51.8%), followed to a lesser extent by ATIII and PS deficiencies that were in 16 (28.6%) and 11 (19.6%) children.

Taking thrombotic risk factors into account, 36 children (64.3%) were at risk of thrombosis, some with a single risk factor and others with several (Table III). Among children at risk of thrombosis, 26 or 72.2% had albumin levels < 20 g/L, 23(63.9%) had fibrinogen > 6 g/L, 11(30.5%) had ATIII < 70% and 22(61.1%) had D-Dimer > 1000 ng/ml (Table III and IV). A significant association was noted between albuminemia < 20 g/L and ATIII (*P=.01),* but not with the other factors used to assess thrombosis risk (Table II).

Table II: Summary table of biological parameters studied.

Parameter	Avg ± SD	Min	Max
Plaquette (/mm^3)	430 800 ± 160 500	125 000	886 000
Albumin (g/L)	23 ± 11,7	4,5	45,9
Fibrinogen (g/L)	5,1 ± 1,9	2,3	8,1
FV (%)	105,8 ± 39,9	17,7	281
FVII (%)	111,1 ± 78,3	20,4	561
FVIII (%)	105,2 ± 67	36,8	454,3
FIX (%)	95,7 ± 32,6	37	232
ATIII (%)	106,5 ± 41,3	18,8	250
PC (%)	192,9 ± 128,7	56,3	936
PS (%)	81,7 ± 17,2	43,6	118,6
D-Dimers (ng/ml)	695 [320, 2260]	100	26 320

Table III: Distribution of children by the thrombotic risk factors.

Risk factor		Frequency	Percentage
No thrombotic risk factor		20	35.7
At least one risk factor		36	64.3
One risk factor		14	25
Changes in	albumin	4	7.1
	fibrinogen	4	7.1
	D-Dimers	6	10.7
Two risk factors		4	7.1
Changes in	albumin/fibrinogen	3	5.3

		albumin/ATIII	1	1.8
		albumin/D-Dimers	2	3.6
	Three risk factors		2	3.6
	Changes in	albumin/fibrinogen/ATIII	7	12.5
		albumin/fibrinogen/D-Dimers	1	1.8
	Four risk factors	Changes in of all parameters	8	14.3
Total			**56**	**100**

Table IV: Study of the association between albuminemia and other thrombotic risk factors in children at risk of thrombosis

Albuminemia (g/L)	Fibrinogen ≤ 6 g/l	Fibrinogen > 6 g/L	Total	*P*
< 20	7	19	26	
≥ 20	6	4	10	*.11*
Total	13	23	36	
Albuminemia (g/L)	ATIII < 70%	ATIII ≥ 70	Total	*P*
< 20	11	5	26	
≥ 20	-	10	10	*.01*
Total	11	25	36	
Albuminemia (g/L)	D-D ≤ 1000 ng/ml	D-D > 1000 ng/ml	Total	*P*
< 20	10	16	26	*1*

≥ 20	4	6	10
Total	14	22	36

4. DISCUSSION

INS is the most common pediatric glomerular disease, affecting 1.15 to 16.9 per 100,000 children per year worldwide [11, 12]. In our study, 56 children with INS were enrolled over the study period.

INS in children is defined as proteinuria more than 50 mg/kg/day associated with hypoalbuminemia less than 30g/L and/or hypoprotidemia less than 60g/L. In our study, hypoalbuminemia was < 30g/L in 35 children (62.5%). These results differ from those found in the study by Tahar G [13], in which all patients had hypoalbuminemia < 20 g/L. This difference could be explained by the fact that some of our children were already on treatment at the time of inclusion in the study, hence the correction of hypoalbuminemia.

The mean platelet count was 430,800 ± 160,500/mm^3 with extremes of 125,000 and 886,000/mm^3. Thrombocytosis, in our study, was present in 29 children or 51.8%. Among these latter, 14 (48.3%) had hypoalbuminemia < 20g/L. During nephrotic syndrome, platelet abnormalities are both quantitative and qualitative: thrombocytosis is found in many cases but is not constant [14]. The work of Aliza [15] showed that in nephrotic syndrome, platelet hyperactivity and hyperaggregability are frequent, and are observed in the presence of various inducers such as adenosine diphosphate, collagen, arachidonic acid, and thrombin, hence the risk of thrombosis. The etiology of this hyperaggregability remains unexplained, with the authors suggesting a probably multifactorial cause (hypoalbuminemia, hyperlipidemia, and hyperfibrinogenemia).

Abnormalities in haemostasis contributing to a thrombophilic state in idiopathic nephrotic syndrome are linked to urinary leakage of small molecular weight molecules and a general

increase in hepatic protein synthesis by entrainment around priority albumin synthesis. There is thus an imbalance between pro-coagulant factors and coagulation inhibitors [16].

In our study, we noted hyperfibrinogenemia > 4 g/L in 34 children, i.e., 60.7%. An increase in FVIII was found in 19.6%, but FV was normal in most children. Data in the literature reported an increase in fibrinogen, FV and FVIII concentrations [14, 17], mainly linked to an increase in their hepatic synthesis. High fibrinogen concentration would increase fibrin complex formation and blood viscosity, and would stimulate platelet aggregation.

We noted normal FVII and IX values in most children. These results are similar to those found in Sagripanti's study [17], in which it was shown that concentrations of vitamin K-dependent factors (II, VII, IX and X) are variable, but most often normal, due to the balance between their increased hepatic synthesis and urinary leakage [17].

A decrease in ATIII was found in 16(28.6%) children in our study. Studies by Citak et al [14] showed that the plasma concentration and activity of antithrombin are generally reduced during nephrotic syndrome, as its urinary leakage is caused by its low molecular weight. This acquired antithrombin deficiency is thought to result in reduced thrombin inhibition, contributing to the hypercoagulable state observed in nephrotic syndrome.

Free PS was reduced in 19.6% of children. Literature data show that total PS concentration is increased in patients with nephrotic syndrome, while its active free form is decreased [18]. This deficiency in free PS is explained by a selective urinary leakage of free PS associated with an increase in PS in a complexed form, as there is an increase in the binding of PS to C4b binding protein, whose hepatic synthesis is increased during nephrotic syndrome [18]. PS activity is therefore ultimately reduced, contributing to the increased risk of thromboembolism [19].

In our study, CP was increased in 31 children (55.3%). It was shown that CP concentration and activity was rising during nephrotic syndrome, as hepatic synthesis of CP was increased, while urinary leakage remained moderate despite its molecular weight close to that of antithrombin [14]. The cause remains unclear.

In our study, D-dimers > 500 ng/ml were found in 35 children (62.5%). These results are in line with the literature, which states that D-dimers, which are products of fibrinolysis, were often but inconsistently high in nephrotic syndrome [20]. This high level, in the absence of clinically significant vascular thrombus, would suggest that there is concomitant formation and lysis of microthrombi without clinical expression.

In our study, thrombotic risk was present in 36 children (64.3%). Among the children at risk of thrombosis, 26 (72.2%) had albumin levels < 20g/L, 23(63.9%) had fibrinogen > 6 g/L, 11(30.5%) had ATIII < 70% and 22(61.1%) had D-Dimer > 1000 ng/ml. Some children had a single risk factor, while others had several associated factors. In our study, all children with ATIII < 70% (11 children) had an albuminemia < 20g/L, showing an increased risk of thrombosis, and a significant association between ATIII < 70% and albuminemia < 20g/L (*P*= *.01*) was found. Plasma antithrombin levels correlate positively with albumin levels [14]. This correlation may be explained by its molecular weight and charge, which are similar to those of albumin [14]. Thromboembolic complications are generally observed when antithrombin levels are <70% [10]. These results are similar to those of Jehanne et al [21], who showed that the major predictive factors of thromboembolic risk in pediatric INS were albuminemia < 25 g/L, the existence of proteinuria and antithrombin < 75%.

5. CONCLUSION

Thrombosis remains an existing complication in idiopathic nephrotic syndrome in children. In our study, the risk of thrombosis was found in 64.3% of children with INS, and the major predictive factor of thrombotic risk was albuminemia < 20 g/L. These results suggest that children with INS should be systematically evaluated for thrombotic risk in the presence of albuminemia < 20g/L.

CONSENT

All the authors state that a written and signed informed consent has been obtained from the patient and/or his/her parents for the use of the data collected for publication purposes. A copy of the written consent is available for review by the editorial office, editor-in-chief and/or members of the editorial board of this journal.

APPROVAL OF THE ETHICS COMMITTEE

This study was approved by the National Ethics Committee for Life Sciences and Health in Ivory Coast.

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