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Different profiles of fatty acids between leukocytes and whole blood in children with idiopathic nephrotic syndrome

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Abstract

Idiopathic nephrotic syndrome (INS) is the most common pediatric glomerular disease, characterized by proteinuria, hypoalbuminemia and edema and caused by an immune dysregulation of T and B cells. Fatty acids (FA) are involved in immune response, with omega-6 prevailing in pro-inflammatory states and omega-3 promoting anti-inflammatory effects. While previous studies of INS assessed FA profile in blood or serum, which may be influenced by many systemic and dietary factors, the intracellular FA metabolism in white blood cells of children with INS, critical to immune cell activation, remains still unexplored. This pilot study compares the FA profile within leukocytes (endo-leukocyte, EL) and whole blood in 35 children with INS and 34 matched controls. INS patients were stratified by steroid sensitivity vs. steroid resistance and by remission vs. proteinuric state. EL FA profiles were analyzed via gas chromatography and dietary habits were evaluated by the Kid Med questionnaire. While blood FA profile of patients demonstrated both elevated omega-6 and omega-3 levels (P-value < 0.005), EL show an inflammatory dominance, with increased omega-6 (P-value < 0.005), but similar omega-3 levels, compared to controls. Furthermore, EL profiles showed reduced saturated FA and palmitic acid but elevated oleic acid levels (P-value < 0.005), possibly indicating a compensatory anti-inflammatory response. This study suggests that EL FA profile may provide unique insights into intracellular mechanisms of inflammation in INS, complementing data arising from blood FA analysis. Despite some limitations, including the small sample size, the study of FA inside the cellular population directly involved in INS underscores its potential in increasing diagnostic precision of FA anomalies in the course of nephrotic syndrome. This new approach may also represent the prerequisite for a clearcut evaluation of the effectiveness of pharmacologic and dietary therapies, like the supplementation with omega 3 metabolites and a diet rich in omega−3.

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Introduction

Idiopathic nephrotic syndrome (INS) is the most common pediatric glomerular disease, characterized by proteinuria, hypoalbuminemia and edema. It is caused by a podocyte damage, which in most cases depends on immunological processes [1, 2]. Tlymphocyte-mediated immunity appears to play a pivotal pathogenetic role in INS. However, even if T-cells may be implicated in INS [3, 4], except for IL–17 A, no distinct T-helper or cytokine profile has been conclusively linked to the disease [5]. Recently, a B lymphocyte-mediated immunity, with the involvement of various dysregulated B cell subsets, has been proposed as a contributing mechanism to the development of INS [5, 6]. In particular, both T and B-cells seem to be involved in the secretion of circulating factors [7] which damage the glomerular filtration barrier, triggering proteinuria and the clinical manifestations of INS.

It has been largely shown that fatty acids (FA) can influence and regulate both inflammation and innate immunity [8]. Among FA, the long chain polyunsaturated FA (LC-PUFA) omega-3 and omega-6 series are the most relevant functional compounds, generating the pro and anti-inflammatory mediators eicosanoids and docosanoids, from arachidonic acid (AA) and DHA respectively. In the course of inflammation, a higher omega-6/omega-3 ratio is related to the predominance of the omega-6 pro-inflammatory compounds over the anti-inflammatory omega-3 LC-PUFA and is influenced by environmental conditions, including diet, intercurrent diseases and drug treatment.

In autoimmune diseases, higher blood omega-6 and AA levels are related to an active inflammatory response via T-helper 2, while higher omega-3 blood levels are associated with a reduced inflammatory state [9, 10]. Although a reverse cause-effect relationship may explain these associations, both experimental and randomized clinical trials in human immune-related disorders have suggested that the modification of FA profile in favor of omega-3 series may contribute to shifting inflammation towards a more favorable course [11].

As regards nephrotic syndrome, as was previously reported that, in children with proteinuria, whole blood LC-PUFA profile was directed towards a pro-inflammatory state [12] and an increased omega-6/omega-3 LC-PUFA ratio was observed, even during remission, indicating a latent, persistent inflammatory state [13].

All the other previous studies of FA profile in kidney diseases either reported a pro-inflammatory status in children with INS [12–15], found an increase of free FA in acute kidney injury [16] or investigated the effect of omega 3 supplementation on proteinuria [17]. All were based, including ours, on blood or serum samples, which may be influenced by the nephrotic syndrome per se. At our knowledge, no data have been published so far on FA profile inside leukocytes (endo-leukocyte, EL, EL FA). While the FA profile in the blood may be influenced by various metabolic factors resulting from proteinuria and endogenous synthesis from essential precursors, the pattern of FA inside leukocytes may more precisely reflect the intracellular mechanisms of INS upstream of proteinuria, as T and B cells were reported as directly implicated in the pathogenesis of the disease.

Therefore, this pilot study aims to compare the FA profile in leukocytes of patients with INS compared with that in the whole blood and in controls.

Patients and methods

Patients

All the patients with INS less than 18 years old who underwent a routine blood test at the Pediatric Nephrology, Dialysis and Transplant Unit of Fondazione Cà Granda IRCCS Ospedale Maggiore Policlinico, Milan, Italy, in the period 1st January – 31th December 2022 were eligible for the study.

All the patients were enrolled with prior parental consensus

Healthy subjects matched for age and gender with neither concomitant inflammatory disease, nor a metabolic or genetic disease, were enrolled as control group, composed of children who performed blood tests in preparation to minor urologic corrective surgery. This control group was selected in order to enrol subjects without any unknown (to the subject) pathologies or inflammatory state that could affect FA profile.

The study protocol was approved by the local ethical committee with document number 0035199-U and was performed following the declaration of Helsinki.

Definition of INS

Idiopathic nephrotic syndrome was defined by the presence of proteinuria > 40 mg/m2/hr in a 24 h urine collection, or a spot urine protein to creatinine ratio (uPr/uCr) of > 2 mg/mg, serum albumin < 2.5 g/dL and edema, in the absence of secondary causes of NS. Patients were categorized into two groups, according to their response to the initial corticosteroid treatment: the steroid sensitive (SS), and the steroid-resistant (SR, subjects who failed to achieve complete remission of proteinuria after 8 weeks of prednisone 60 mg/m^2/day) groups. This last group included cases with either subsequent response to immunosuppressive (IS) drugs or multi-drug resistant patients. Definitions of SS and SR were applied to INS patients according to international guidelines [18].

Treatment

The initial standard medication for the treatment of INS was in all patients oral prednisone (PDN), administered according to the Italian Society of Pediatric Nephrology guidelines [19].

IS drugs were prescribed at standard dosages [19] to steroid dependent and steroid resistant patients and consisted of tacrolimus (Fk) and/or mycophenolate mofetil.: tacrolimus (Fk) starting dose was 0.1–0.2 mg/kg/day (maximum dose 10 mg) in 2 doses, to achieve trough blood levels of 3–7 ng/mL; mycophenolate mofetil starting dose was 1200 mg/m2 body surface area (maximum dose 3000 mg/day), divided into two oral doses every 12 h. Subsequent dosages of both drugs were administered based on drug blood levels.

Before and during the administration of corticosteroid and IS drugs, patients received support treatment, either with furosemide, spironolactone or hydrochlorothiazide. When necessary, also one or more of the following antihypertensive drugs were prescribed: ACE-inhibitors, beta blockers and calcium antagonists.

Dietary data

As blood FA profile is influenced by dietary supply, the exogenous FA intake was assessed in all the patients based on the criterium of adherence to Mediterranean diet, through the validated Kid Med questionnaire [20] filled in by the parents, which explores the balance between intake of saturated and unsaturated FA and omega-6 and omega-3 FA series. It consists of 16 items on eating habits, like regular fish and daily fresh vegetables consumption or junk food consumption, each to be answered Yes or No. The minimum value of the resulting score is -4 (low adherence), and the maximum value is + 12 (high adherence).

Blood sample collection and leukocytes separation

Three ml of blood were collected and centrifuged with histopaque-1077 (Sigma Aldrich, St. Louis Missouri, USA) to separate leukocytes. Three ml of histopaque-1077 were transferred to a 15 mL centrifuge tube; 2 mL of blood were mixed with 2 mL of physiological saline solution. The diluted blood was carefully layered over the histopaque, without mixing. The tube was centrifuged at 400 G at room temperature for 15 min. The first layer of plasma was removed, and leukocyte layer plus histopaque was collected in a new tube. Leukocytes were washed with physiological saline solution by centrifugation at 160 G at room temperature.

Fatty acids analysis

Isolated leukocytes and 50 µL of whole blood were methylated with 800 µl of HClMe 3 N (Sigma Aldrich, St Louis Missouri, USA), incubated for 1 h at 90 °C and then refrigerated ad 4 °C for 10 min. Afterwards, 2 mL of KCl solution (Sigma Aldrich, St Louis Missouri, USA) and 330 µL hexane (Sigma-Aldrich St Louis Missouri, USA) were added. Samples were then vortexed and then centrifuged at 3000 rpm for 10 min. Finally, hexane layer (the upper layer) was collected from each vial and transferred into a gas chromatography vial for FA profile evaluation with gas-chromatographer Shimadzu Nexis GC-2030 (Shimadzu, Kyoto Japan) equipped with a 30 m fused silica capillary column FAMEWAX Restek (Restek Bellefonte, Pennsylvania, USA). Gas chromatography results were analyzed using Labsolution software v. 5.97 SP1 (Shimadzu, Kyoto Japan).

The relative percentage of total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), total omega-6, total omega-3, omega-6/omega-3 ratio and all single FAs were considered in the analysis.

Biochemical analyses

Urinary protein (uPr), urinary creatinine (UCr), blood white cell count, serum triglycerides, total cholesterol and HDL cholesterol were measured and the uPr/uCr ratio was calculated, as part of patients' routine check analyses.

Statistical analysis

After a Shapiro-Wilk test to check the sample normality distribution (for each FA) the appropriate statistic test (T-test for normal distribution or Kruskal Wallis for not normal distribution) was used. In all tests a P-value < 0,05 was considered significant. T-test and Kruskal Wallis statistical tests were performed with software SPSS v. 21 (IBM, Armonk, New York, USA) while Shapiro-Wilk test was performed with software PAST (Oslo University, Oslo, Norway).

Sample size

To achieve a statistical significance level of 0.05 and a power of 80% in the statistical tests, a minimum cohort of 26 patients and an equal number of healthy controls was calculated.

This calculation was based on the mean and standard deviation of fatty acid values in pediatric nephrology patients derived from the literature [12] and corresponding data obtained from individuals referred to the pediatric urology unit, already included in another study approved by the ethics committee as healthy controls.

The cohort size determination was carried out by evaluating data related to oleic acid (18:1n9), linoleic acid (18:2n6), arachidonic acid (20:4n6), EPA (20:5n3), and DHA (22:6n3), considering the highest value among the five groups as the minimum cohort size.

Table 1 Patients' demographic, clinical and biochemical data (mean ± SD)

Age (years)	**11,8 ± 4,9**
M/F	17/18
UPr/UCr (mg/mg)	0,71 ± 0,94
Leukocytes (109/l)	2,82 ± 0,91
Total protein (g/dl)	6,33 ± 0,82
Serum albumin (g/dl)	4,13 ± 0,79
Triglycerides (mg/dl)	101,48 ± 57,02
Cholesterol (mg/dl)	277,6 ± 527,25
HDL cholesterol (mg/dl)	62,72 ± 15,86
Patients on Fk (number)	12
Patients on MMF (number)	17
Patients on Fk and MMF (number)	6

UPr: urinary protein; UCr: urinary creatinine; Fk: tacrolimus; MMF: mycophenolate mofetil

The calculation was performed using the PS Power and Sample Size Calculation software version 3.0 (Vanderbilt Biostatistics).

Results

Thirty-five children with INS (age 11,8 ± 4,9 years, 17 males), 20 SS and 15 SR, were enrolled into the study. Among SS patients, five were in relapse and 15 in remission at the time of blood sampling. Six out of the 15 SR cases were multi-drug resistant and maintained persistent proteinuria, while 9 were in remission after IS treatment. Thirty four children served as control group.

Demographic data, mean clinical and biochemical data, white blood cells sorting and data on IS treatment are summarized in Table 1.

The average Kid med score was 5.03 ± 2.2 (median value 7), indicating a sub-optimal adherence to Mediterranean diet in our population. No differences in the Kid med score were found among the patients' groups.

Blood fatty acid analysis

Table 2 shows blood FA values in SS and SR patients, each of them divided in relation to the state of proteinuria or remission, and in controls.

Regarding single fatty acids, DHA was higher in all patients' sub-groups than in controls, while AA was higher only in SS patients on remission. In addition, palmitic acid (16:0) was lower in all four sub-groups than in controls, while linoleic acid (18:2n6, LA) was higher only in SR patients on remission, respectively.

Considering FA groups, PUFA levels were higher in all patients' sub-groups compared to controls; SFA levels were significantly lower in the SS on remission, SS with proteinuria and SR with proteinuria sub-groups compared to controls and MUFA values were lower only in SS patients on remission.

Omega-6 levels were higher in all sub-groups compared to controls, with the exception of SR patients with proteinuria. Omega-3 levels were also higher in all patients'

Table 2 Blood fatty acid profile of the four patients' sub-groups compared to controls

	SS (remission)	**SR (remission)**	**SS (proteinuria)**	**SR (proteinuria)**	**Controls**
16.0[A]	**22.76 ± 1.54****	**20.09 ± 0.94***	**22.13 ± 1.66****	**21.27 ± 1.53*****	24.63 ± 1.76
16:1n9[A]	0.86 ± 0.29	0.81 ± 0.30	0.89 ± 0.23	0.94 ± 0.19	0.76 ± 0.35
18:0[A]	11.97 ± 1.47	10.81 ± 2.11	**11.06 ± 1.59***	**9.72 ± 0.39*****	12.85 ± 1.45
18:1n9[B]	**16.69 ± 2.62****	20.04 ± 4.11	18.38 ± 1.84	22.17 ± 2.02	20.55 ± 3.64
18:1n7[B]	1.36 ± 0.29	1.23 ± 0.32	1.31 ± 0.17	1.33 ± 0.27	1.75 ± 1.88
18:2n6 (LA)[B]	22.78 ± 4.61	**24.35 ± 2.91****	26.92 ± 5.51	24.76 ± 4.34	21.82 ± 3.19
18:3n3 (ALA)[B]	0.19 ± 0.15	0.22 ± 0.06	0.25 ± 0.06	0.25 ± 0.07	0.2 ± 0.13
20:3n6[B]	1.61 ± 0.38	**1.73 ± 0.63***	1.55 ± 0.53	1.49 ± 0.28	1.43 ± 0.27
20:4n6 (AA)[A]	**11.64 ± 2.32*****	10.16 ± 2.42	9.16 ± 1.81	9.83 ± 0.35	9.34 ± 1.65
20:5n3 (EPA)[B]	0.21 ± 0.15	0.28 ± 0.19	0.16 ± 0.07	0.22 ± 0.14	0.24 ± 0.21
22:0[B]	1.72 ± 2.44	0.99 ± 0.28	1.20 ± 0.17	1.09 ± 0.14	1.08 ± 0.22
22:5n3[A]	**0.75 ± 0.19*****	**0.72 ± 0.17*****	0.50 ± 0.13	0.51 ± 0.12	0.50 ± 0.19
24:0[A]	**2.16 ± 0.68****	1.61 ± 0.65	1.73 ± 0.34	1.61 ± 0.29	1.65 ± 0.46
22:6n3 (DHA)[A]	**2.68 ± 0.85*****	**2.21 ± 0.98****	**2.35 ± 0.43****	**2.58 ± 0.83****	1.52 ± 0.61
24:1[A]	**2.34 ± 0.77****	1.57 ± 0.78	1.71 ± 0.52	1.91 ± 0.30	1.73 ± 0.51
PUFA[A]	**39.87 ± 3.06*****	**39.67 ± 3.73****	**40.88 ± 3.81****	**39.64 ± 3.17***	35.06 ± 3.84
MUFA[B]	**21.24 ± 2.88****	23.66 ± 4.20	22.29 ± 1.91	26.35 ± 2.08	24.79 ± 3.85
SFA[A]	38.6 ± 3.4	**36.49 ± 2.10****	**36.12 ± 3.29****	**33.69 ± 1.89*****	40.22 ± 2.72
omega-3[B]	**3.83 ± 0.97*****	**3.43 ± 1.22****	3.26 ± 0.47	**3.56 ± 0.99***	2.47 ± 0.87
omega-6[B]	**36.03 ± 3.16****	**36.24 ± 3.38*****	**37.62 ± 4.22***	36.08 ± 3.96	32.59 ± 3.72
AA/DHA[B]	**4.84 ± 2.06****	5.71 ± 3.3	**3.88 ± 0.11****	**4.10 ± 1.17***	6.91 ± 2.45
omega-6/omega-3[B]	**10.16 ± 3.38****	12.21 ± 5.62	11.91 ± 3.31	11.05 ± 4.15	15.03 ± 6.51

In bold*P*-values: * < 0,05. ** <0,005, *** < 0,0005 vs. CTRL at (A) T-test for samples with normal distribution or (B) Kruskal Wallis test in case of not normal distribution

sub-groups than in controls, except for SS patients with proteinuria,.

The pro-inflammatory indices AA/DHA and omega-6/omega-3 ratios were lower in SS patients on remission, while only patients with proteinuria (both SS and SR) showed a higher AA/DHA ratio compared to controls.

Endo-leukocyte fatty acid analysis

The FA profile inside leukocytes is shown in Table 3. Regarding single FA, DHA and AA did not differ in patients compared to controls. In contrast, LA and alpha-linolenic acid (18:3n3, ALA) showed some differences, respectively. LA levels were higher in patients than in controls, except for SR patients with proteinuria, while ALA levels were higher in SR patients on remission and SS patients with proteinuria. In addition, palmitic acid (16:0) and stearic acid (18:0) were lower, whereas oleic acid (18:1n9) higher, in patients than in controls.

Considering FA groups, there were higher levels of PUFA, but lower SFA levels, in all patients' sub-groups than in controls. MUFA resulted higher than in controls only in SR patients on remission.

Omega-6 levels were higher in all four sub-groups, while omega-3 showed no significant differences compared to controls. Accordingly, the pro-inflammatory indices AA/DHA and omega-6/omega-3 were not different between patients and controls.

Blood fatty acids vs. Endo-leukocyte fatty acids

The main differences between blood and EL FA profiles of patients' sub-groups compared to controls were observed for MUFA, omega-3, pro-inflammatory indices and all single FA, except palmitic acid (Fig. 1). Concordances were found only for PUFA, SFA, omega-6 and palmitic acid. On the whole, most differences between patients and controls were detected in the blood FA profile, compared to the pattern within leukocytes.

Discussion

This pilot observational study highlights several differences between whole blood and endo-leukocyte FA profiles. In line with a previous study in adult healthy subjects [21], blood FA profile differs when compared to the pattern inside leukocytes. In addition, our study confirms that children with INS have a different FA profile in the whole blood compared to that of controls, strengthening the value of FA profile within leukocytes in sustaining inflammation in the course of INS [12, 13].

The FA pattern in a distinct cell pool (such as leukocytes) compared to another (whole blood, plasma, serum) may help to disentangle the specific FA roles in different diseases, adding information to pro-inflammatory mechanisms. Indeed, alterations in the FA profile have been found not only during proteinuria, but also on remission [12, 13].

Table 3 Endo-leukocyte fatty acid profile of the patients' sub-groups compared to controls

	SS (remission)	SR (remission)	SS (proteinuria)	SR (proteinuria)	Controls
16.0[A]	**21.73 ± 1.56*****	**21.41 ± 1.52*****	**21.90 ± 1.53*****	**20.94 ± 2.10*****	24.34 ± 1.21
16:1n9[A]	**0.43 ± 0.28***	0.40 ± 0.18	0.43 ± 0.31	0.46 ± 0.07	0.30 ± 0.15
18:0[A]	**16.10 ± 2.27***	**15.46 ± 3.31****	**13.82 ± 2.67*****	**15.87 ± 1.61***	17.50 ± 1.06
18:1n9[B]	**16.04 ± 2.36***	**17.30 ± 4.15****	**16.24 ± 2.92***	**18.39 ± 1.10****	14.19 ± 1.98
18:1n7[B]	1.31 ± 0.19	1.08 ± 0.20	1.13 ± 0.18	1.39 ± 0.04	1.11 ± 0.41
18:2n6 (LA)[B]	**13.47 ± 4.17***	**13.68 ± 4.18****	**16.10 ± 4.25*****	12.80 ± 3.97	11.19 ± 1.69
18:3n3 (ALA)[B]	0.10 ± 0.4	**0.14 ± 0.06****	**0.15 ± 0.12***	0.09 ± 0.01	0.08 ± 0.05
20:3n6[B]	1.70 ± 0.49	2.55 ± 3.44	1.59 ± 0.46	1.44 ± 0.25	1.69 ± 0.32
20:4n6 (AA)[A]	17.21 ± 3.05	16.01 ± 3.30	16.86 ± 3.39	17.82 ± 2.31	15.69 ± 2.23
20:5n3 (EPA)[B]	0.26 ± 0.27	0.35 ± 0.32	0.20 ± 0.09	0.11 ± 0.09	0.27 ± 0.17
22:0[B]	1.76 ± 1.12	1.53 ± 0.85	1.87 ± 2.12	**1.08 ± 0.97***	1.55 ± 0.30
22:5n3[A]	1.09 ± 0.22	1.41 ± 0.56	1.25 ± 1.01	1.14 ± 0.16	1.13 ± 0.35
24:0[A]	**2.53 ± 1.05*****	3.35 ± 2.73	**2.21 ± 0.78*****	**2.25 ± 0.81***	4.05 ± 1.22
22:6n3 (DHA)[A]	3.18 ± 1.06	2.51 ± 0.69	3.51 ± 1.34	3.32 ± 0.60	2.86 ± 1.03
24:1[A]	**2.71 ± 1.09****	**2.53 ± 0.79****	**2.52 ± 0.97****	2.71 ± 0.75	3.59 ± 0.78
PUFA[A]	**37.01 ± 3.18*****	**36.66 ± 6.25****	**39.65 ± 2.96*****	**36.72 ± 2.40***	32.91 ± 2.59
MUFA[B]	20.50 ± 2.61	**21.32 ± 3.66***	20.31 ± 3.17	22.95 ± 1.23	19.19 ± 1.93
SFA[A]	**42.12 ± 3.69*****	**41.75 ± 4.38*****	**39.80 ± 3.51*****	**40.13 ± 1.20*****	47.44 ± 2.16
omega-3[B]	4.63 ± 3.69	4.41 ± 0.81	5.10 ± 2.18	4.66 ± 0.52	4.34 ± 1.33
omega-6[B]	**32.38 ± 3.2*****	**32.25 ± 5.98****	**34.55 ± 3.71*****	**32.06 ± 1.89***	28.57 ± 2.34
AA/DHA[B]	5.95 ± 2.03	6.89 ± 2.48	5.27 ± 1.77	5.57 ± 1.66	6.10 ± 2.05
omega-6/omega-3[B]	7.53 ± 2.38	7.44 ± 1.68	7.76 ± 2.73	6.91 ± 0.41	7.31 ± 2.76

In bold*P*-values: * < 0,05. ** <0,005, *** < 0,0005 vs. CTRL at (A) T-test for samples with normal distribution or (B) Kruskal Wallis test in case of not normal distribution

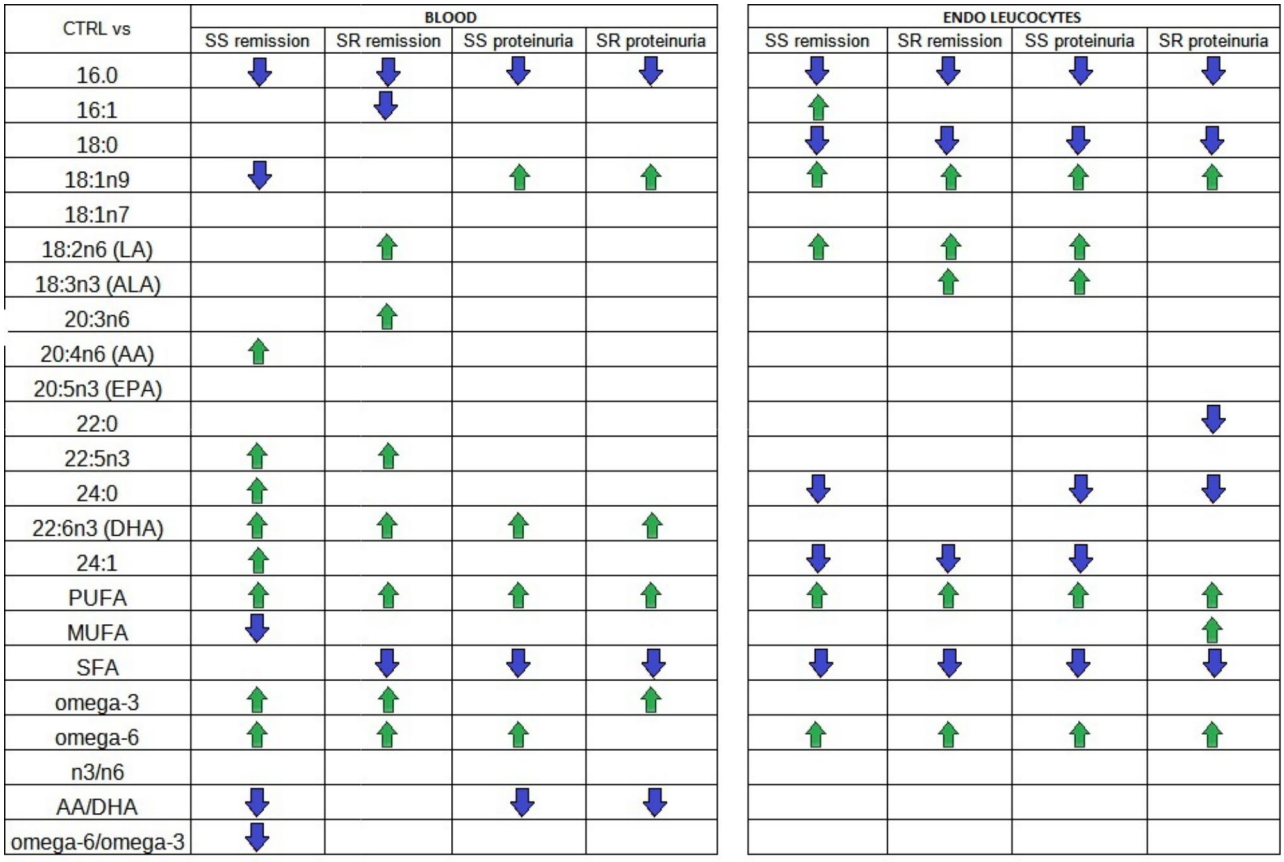

Fig. 1 Blood and endo leukocyte FA profiles of patients compared to controls

Pro-inflammatory omega-6 and anti-inflammatory omega-3 blood levels were higher in patients than in controls, at difference with expectations. An increased FA biosynthesis, decreased FA metabolism, or a combination of both, might be at the origin of this finding in the FA profile of circulating FA pools. On the contrary, EL profile provided more informative insights into inflammation. Indeed, higher levels of pro-inflammatory omega-6 in the EL FA profile of patients compared to controls, with no differences in anti-inflammatory omega-3 levels, have been found. Accordingly, the EL FA pattern, free of potential confounding factors present in circulating pools, might indicate an active role of lymphocytes in sustaining a pro-inflammatory state in INS.

This pro-inflammatory pattern is in accordance with previous findings of the literature [12, 13].

A further, so far unpublished, result concerns the lower levels of SFA and palmitic acid in patients compared to controls, both in whole blood and inside leukocytes. High palmitic acid and SFA levels correlate with the increase of LDL cholesterol, via LDL receptors [22], and it is well known that hypercholesterolemia is one of the main events that occur during INS. Further studies on the levels of large and small LDL particles in INS patients could help to elucidate this point [23, 24].

Finally, the lower level of stearic acid (18.0) and the higher level of oleic acid (18:1n9) inside leukocytes are consistent with the findings on PUFA. Oleic acid is directly synthetized from stearic acid and is described as having anti-inflammatory actions, playing a role in the activation of different pathways and differentiation of T-cells, contrasting arachidonic acid pro-inflammatory action [25, 26].

It is well known that blood FA levels are influenced by their dietary intake. There are various methods to assess it, such as dietary interviews and, more recently, the KidMed score, each with its own limitations [27, 28]. As regards dietary interview, dieticians are well aware of the difficulties in assessing the exact amount of each FA from every food item provided by the family or school canteen to the child, due to the huge number of foods on the market, in addition to the approximation in the quantities weighed [28]. This is the reason why the use of the KidMed score, a validated method to assess adherence to the Mediterranean diet and, consequently, the ratio between saturated and unsaturated fatty acids, has gained ground. The Kidmed score has also the advantage, compared to dietary interview, of providing information about the balance between omega-6 and omega-3 FA series [29]. Since no statistically significant differences were observed in

the Kidmed score results among the studied cohorts, so is possible to conclude that diet was not the determinant factor in the differences found in the various FA profiles.

Strengths and limitation

Strong points of our study are the precise characterization of the patients, including their dietary FA consumption, which is a relevant environmental factor influencing blood FA profile, and, most of all, the selective analysis of FA inside immune cells.

A limitation of our study is the small cohort size, particularly if subgroups are considered, as it was designed as an initial exploratory single-center pilot study. Nevertheless, these preliminary observations suggest that further well-powered studies, that include FA metabolites, particularly eicosanoids and resolvins, are warranted.

Conclusion

In conclusion, the novel approach of analysing FA pattern inside leukocytes may represent an added value to the study of INS, more accurately reflecting the endo-cellular FA metabolism and its possible link with immune activity, as T and B cells are populations directly involved in the pathogenesis of INS.

While the FA profiles of circulating pools of FA in blood and plasma may represent a more easily available and cheaper approach, matching their patterns with others on specific tissues or cell populations may increase diagnostic precision of FA anomalies in the course of nephrotic syndrome, contributing to better elucidate FA-mediated immunological mechanisms. The availability of this new diagnostic methodology represents the prerequisite for a precise evaluation of the effectiveness of pharmacologic and dietary therapies, like the supplementation with omega 3 metabolites, the treatment with inhibitors of cyclooxygenase and lipooxygenase and, most of all, a diet rich in omega-3.

Author contributions

S.T. and A.E. wrote the main manuscript text and E.D and A.B select and provided controls subjects. All authors reviewed the manuscript.

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Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Ethical approval

The study protocol was approved by the local ethical committee with document number 0035199-U and was performed following the declaration of Helsinki. All subjects consent to participate at the study and consent to the results publication.

Informed consent statement

All patients, or their tutors, signed informed consent to participate in this study as specified in the ICMJE recommendations.

Competing interests

The authors declare no competing interests.

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