



# Circulating plasmablasts in children with steroid-sensitive nephrotic syndrome

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## Abstract

**Background** The therapeutic efficacy of B cell-depleting anti-CD20 treatment in both pediatric and adult steroid-sensitive nephrotic syndromes (SSNS) suggests that B cells play a pathogenic role in the disease. In adults with minimal change disease (MCD), only circulating plasmablasts are increased during the active phase of the disease, among B cell subsets. These cells have not been studied yet in children with SSNS.

**Methods** We retrospectively quantified by flow cytometry analysis circulating plasmablasts in 107 pediatric patients with SSNS (51 at disease onset, 27 during relapse, and 29 in remission). Data were compared with an equal number of age- and sex-matched healthy donors (HD).

**Results** Circulating plasmablast levels, expressed as percentage of total CD19<sup>+</sup> B cells or as percentage of total lymphocytes, were normal in all SSNS subgroups, compared to HD. Patients in remission had significantly fewer circulating plasmablasts compared to patients at disease onset. No significant correlation was observed between plasmablast levels and proteinuria or serum proteins, at onset. Treatment with prednisone and mycophenolate mofetil significantly reduced circulating levels of plasmablasts, unlike treatment with prednisone and calcineurin inhibitors.

**Conclusions** The B cell phenotype of children with SSNS differs from that of adults with MCD. This may justify different therapeutic approaches.

**Keywords** B cells · Pediatric nephrology · Immunosuppression

## Introduction

A pathogenic role of B cells in idiopathic nephrotic syndrome is suggested by the therapeutic efficacy of B cell-depleting anti-CD20 monoclonal antibodies in children and adults [1, 2]. Several alterations in B cell homeostasis have been reported in children with steroid-sensitive nephrotic syndrome (SSNS). In particular, an increase in total CD19<sup>+</sup>, transitional and memory B cells has been observed at disease onset and during relapse; these alterations can be reversed by immunosuppressive therapies [3–5]. Recently, the phenotype of circulating B cells has been studied in adult patients with minimal change disease (MCD) [6]. Compared to healthy controls, only circulating plasmablasts during the active phase of the disease were significantly increased and returned to normal levels after remission [6]. Plasmablasts are short-lived activated B cells that migrate from germinal centers to peripheral blood and later differentiate into antibody-producing plasma cells [7].

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Federica Zotta and Marina Vivarelli contributed equally to the study

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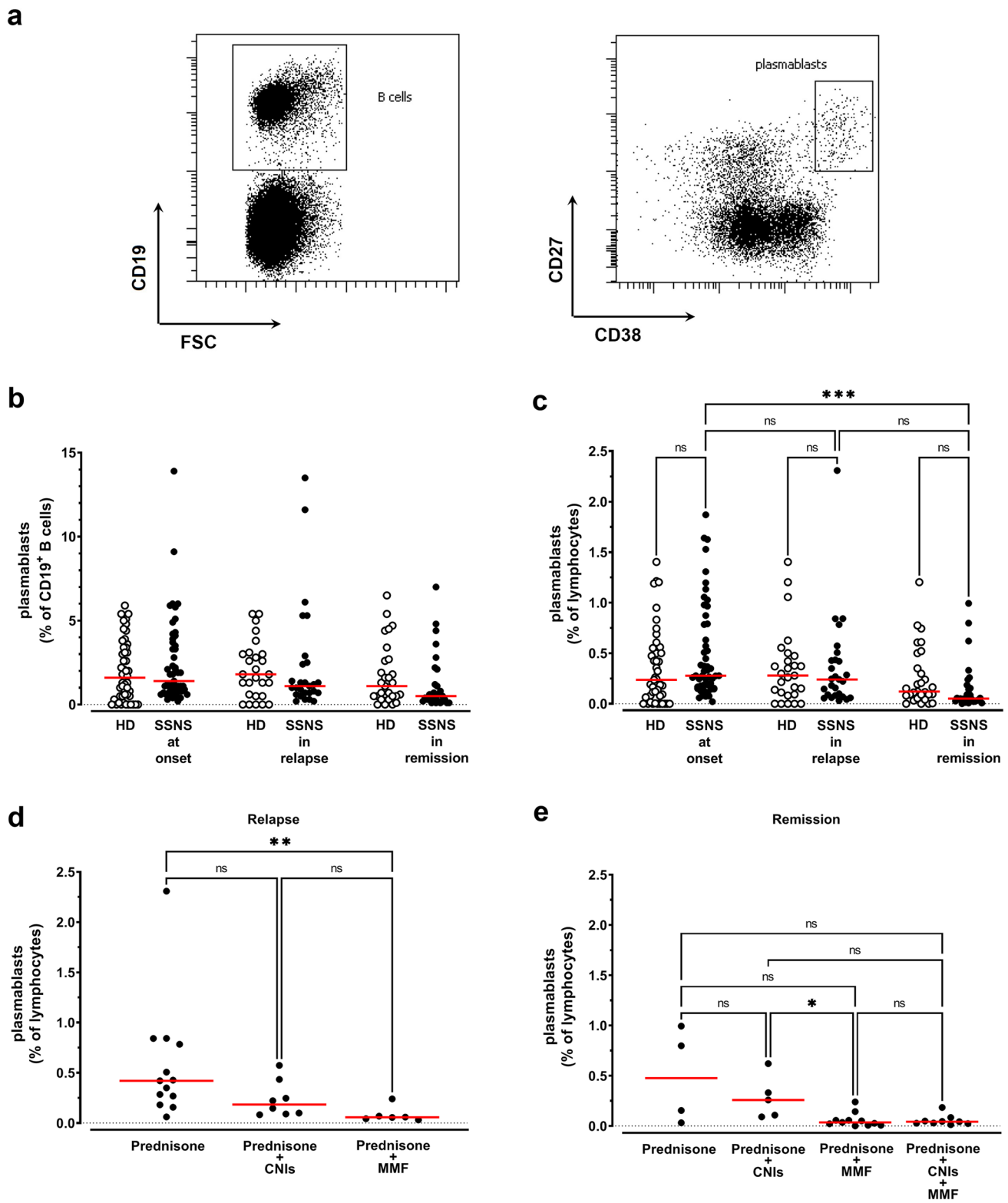
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Herein, we have measured circulating plasmablast levels in a large cohort of children with SSNS and an equal number of age- and sex-matched healthy donors (HD).

**Fig. 1** Multicolor flow cytometry analysis of circulating plasmablasts in pediatric steroid-sensitive nephrotic syndrome (SSNS). Levels of plasmablasts from SSNS patients at disease onset ( $n=51$ ), in relapse ( $n=27$ ), or in remission ( $n=29$ ) were compared with values of the same number of age- and sex-matched healthy donors (HD). **a** Gating strategy to identify plasmablasts by multicolor flow cytometry. **b** and **c** Circulating plasmablasts expressed as percentage of **b** total CD19<sup>+</sup> B cells or **c** total lymphocytes were shown. **d** and **e** Circulating plasmablast levels expressed as percentage of total lymphocytes from SSNS in **d** relapse or in **e** remission stratified according to immunosuppression received in the previous 6 months. CNIs, calcineurin inhibitors; MMF, mycophenolate mofetil. Horizontal red lines indicate the medians. Differences between groups were compared using the non-parametric Kruskal–Wallis test and, if significant, pairwise comparisons were evaluated by the Dunn’s multiple comparisons test. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$

## Patients and methods

### Study population

The present study is a monocentric retrospective analysis on pediatric patients with SSNS followed at the Bambino Gesù Children’s Hospital – IRCCS in Rome. The study was approved by the local ethics committee and complies with the declaration of Helsinki. The cohort ( $n=107$  patients with SSNS and  $n=107$  age- and sex-matched HD) has already been described (see reference [4] for complete description of patient characteristics and immunosuppressive treatments). Nephrotic syndrome, steroid sensitivity, relapse, and remission were defined as previously described [8]. Overall, 51 patients were tested at disease onset before receiving immunosuppressive therapy, 27 during relapse, and 29 in remission for at least 1 month. SSNS patients in the different phases of disease are not the same patients, as previously reported [4].

### Flow cytometry

Staining of thawed peripheral blood mononuclear cells with fluorochrome-conjugated antibodies was already performed in the previous report [4]. In the current study, data have been re-evaluated by flow cytometry analysis (FACSDiva Software, BD Biosciences) to determine the amount of circulating plasmablasts, identified as CD27<sup>+</sup>CD38<sup>high</sup> cells gated in CD19<sup>+</sup> total B cells (Fig. 1a).

### Statistical analyses

All analyses were performed with the GraphPad Prism 9 software (San Diego, CA). Continuous data are expressed as median values and interquartile range ([IQR]). Values were compared with the Kruskal–Wallis test. If significant, further pairwise comparisons were performed with the Dunn’s multiple comparisons test. Linear regression was used for

correlation analyses. *P* values are two-sided and considered significant for  $p < 0.05$ .

## Results

Circulating plasmablasts, expressed as percentage of total CD19<sup>+</sup> B lymphocytes, were similar between all subgroups of patients and between patients and HD, and no significant difference was found by Kruskal–Wallis test (Fig. 1b). Median levels of circulating plasmablasts of SSNS patients were 1.4% [0.7–3.7] at onset, 1.1% [0.7–2.5] during relapse, and 0.5% [0.2–1.7] in remission. When plasmablasts were expressed as % of total lymphocytes, no significant difference was observed between SSNS patients and HD (Fig. 1c). However, a significant difference was found in circulating levels of plasmablasts by comparing SSNS patients at onset vs. SSNS patients in remission ( $p < 0.001$ ; Fig. 1c). No significant correlation was observed between plasmablast levels and proteinuria or serum proteins at onset ( $r = -0.01$ ,  $p = 0.94$  and  $r = -0.05$ ,  $p = 0.74$ , respectively).

Patients in relapse or in remission were further divided based on the immunosuppressive treatment that they had received in the previous 6 months. Although the number of patients was limited, children treated with a combination of prednisone and mycophenolate mofetil (MMF) had significantly lower levels of circulating plasmablasts (Fig. 1d and e). Of note, this was not observed in patients treated with prednisone and calcineurin inhibitors (CNIs) (Fig. 1d and e).

## Discussion

While the therapeutic efficacy of B cell-depleting anti-CD20 treatments has been well demonstrated in both children and adults [1, 2], the identification of the pathogenic B cell subset(s) driving SSNS is still elusive. We previously reported increased levels of total CD19<sup>+</sup>, transitional and memory B cells in children with SSNS at disease onset before immunosuppressive treatment [4]. We have also observed that only memory B cells remained significantly increased during relapse, despite immunosuppression therapies with prednisone and MMF or CNIs; conversely, memory B cells normalized during remission only [4]. These results have also been recently confirmed by Ling et al. [5]. Taken together, they support the evidence of a higher risk of relapse in children with early reappearance of memory B cells after anti-CD20 therapy [9, 10].

Until recently, similar studies have not been carried in adults with SSNS. A recent study by Oniszczuk et al. has characterized the B cell phenotype in adults with MCD or with idiopathic membranous nephropathy (IMN) [6].

The authors observed that, among all the B cell subsets, only plasmablasts were significantly increased compared to healthy controls during the active phase of disease and underwent normalization during remission. Moreover, plasmablast levels correlated positively with proteinuria and the degree of hypoalbuminemia [6]. Based on these findings, we have tested if similar results also apply to a cohort of children with SSNS and of age- and sex-matched HD that we have previously described [4]. In contrast to adult MCD, we observed that circulating levels of plasmablasts were normal (comparable to those of HD) in children with SSNS at all stages of disease (onset, relapse, remission) and did not correlate with the severity of nephrotic syndrome at onset. In addition, we observed that SSNS pediatric patients in remission had reduced circulating plasmablasts compared to patients at onset before starting immunosuppression (but not to patients in relapse who were receiving immunosuppressive drugs), likely reflecting the immunomodulatory effects exerted by the administered immunosuppression more than a pathogenic role of plasmablasts in pediatric SSNS patients. Accordingly, plasmablast levels are reduced by MMF more than memory B cells [7], as also observed in our cohort, whereas CNIs do not exert a direct effect [11].

Oniszczyk et al. also found increased serum levels of B cell-activating factor (BAFF) in both MCD and IMN patients during active disease. BAFF serum concentrations correlated positively with plasmablast levels in MCD patients [6], supporting the role of BAFF in the terminal differentiation of B cells into plasmablasts and plasma cells [12]. Based on these results, they hypothesized that belimumab, an anti-BAFF monoclonal antibody shown to be effective in systemic lupus erythematosus and in IMN [13, 14], may represent a potential therapy for MCD in adults. Of note, we recently performed a pilot study evaluating the safety and efficacy of short-term belimumab treatment in a small cohort of SSNS children and failed to observe evidence of improvement [15], corroborating the idea that plasmablasts do not play a key pathogenic role in pediatric SSNS.

Taken together, these results suggest differences in the alterations of B cell homeostasis observed during SSNS in children and in adults. Children are known to respond much more rapidly to prednisone than adults [8]. Potentially, these differences could in part depend on the B cell subset that is implicated in the pathogenesis of the disease, namely, memory B cells in children, which respond rapidly to prednisone, and plasmablasts in adults, which require a more prolonged treatment [7].

This study has limitations: in particular, it has a retrospective and cross-sectional design and the sub-analyses included a limited number of patients. Nonetheless, we observed striking differences between children and adults.

If confirmed, our results can support the notion that MCD in children and adults have different pathogenesis and may require different therapeutic approaches.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Code availability** Not applicable.

## Declarations

**Ethics approval** The study was approved by Bambino Gesù Children's Hospital Ethics Committee and was conducted in compliance with the declaration of Helsinki.

**Consent to participate** Written informed consent on behalf of the minors/children enrolled was obtained from parents.

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare that they have no conflict of interest.

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