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Letter to the Editors-in-Chief

Absolute immature platelet count dynamics of thrombotic thrombocytopenic purpura patients with high ADAMTS13 inhibitor

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Acquired/idiopathic thrombotic thrombocytopenic purpura (TTP) is manifested by auto-antibody formation to ADAMTS13 which leads to accumulation of ultra-large molecular weight von Willebrand Factor multimers, thrombocytopenia, and diffuse microvascular thrombi [1]. A recent report has given support that a TTP diagnosis should be limited to patients with ADAMTS13 deficiency secondary to autoantibody formation who mostly respond to therapeutic plasma exchange (TPE) [2].

At many institutions ADAMTS13 testing is a send-out test often requiring TPE initiation before this information is available. Therefore, other tests that complement diagnosis and/or provide information of response to therapy are needed. Immature platelets are newly-released from bone marrow and are larger in size with greater RNA content than mature platelets [3,4]. Immature platelet counts are obtained as a fraction of total platelet count (immature platelet fraction [%-IPF]), from which the absolute immature platelet count (A-IPC) can be derived [4]. A high %-IPF can indicate thrombocytopenia due to platelet consumption or recovery from thrombocytopenic etiologies [5], while low %-IPF indicates bone marrow suppression [6].

A-IPC has been suggested to be a better gauge of thrombocytopenia in immune thrombocytopenic purpura [7], in heparin-induced thrombocytopenia [8], and in TTP [9]. In the latter a 3-fold ratio increase in A-IPC from baseline (day of presentation) after TPE initiation has been reported [9]. This test can be obtained without delaying patient care. Importantly, ADAMTS13 deficiency can occur with a high inhibitor that can make treatment protracted requiring aggressive therapy for disease remission [10]. Due to therapy difficulties in these patients, timing of immunosuppression may also need to be adjusted to hasten recovery [11]. Therefore, we aimed to determine A-IPC dynamics of TTP patients with high inhibitor in response to therapy.

Eight TTP patients with low ADAMTS13 activity (< 5%) and high inhibitor (> 1 IU, Table 1) treated between 01/01/2014 and 7/30/2018 at our facility, a > 1000-bed tertiary academic medical referral center were selected. Patients presented with thrombocytopenia (platelet count [PLT] < 30×10^9/L), microangiopathic-hemolytic-anemia (MAHA), acute kidney injury, and/or mild neurological deficits. ADAMTS13 testing obtained prior to first TPE initiation was resulted post initiation. Clinical information such as comorbidities, medications, transfusion reaction history, and time to recovery were obtained. Laboratory information for cohort is presented in Table 1.

All ADAMTS13 samples were sent-out to the Blood Center of Wisconsin [9]. Clinical response was defined as PLT of at least 150×10^9/L for at least 2 days and used to guide TPE discontinuation. Study was approved by our institutional review board.

Procedures consisted of exchanges of 1–1.5 plasma volumes using ABO type-specific plasma as described [9,12]. Complete blood count (CBC) was performed using an automated hematology analyzer (Model XN-9000, Sysmex America, Inc., Mundelein, IL) [4]. %-IPF was obtained with pre-TPE CBC and used to derive A-IPC by multiplying the fraction times absolute optical platelet count [4]. A-IPC ratio was calculated using A-IPC prior to first TPE as denominator. PLT and A-IPC changes after up to 5 TPE treatments [9] were analyzed as potential indicators of TPE response which corresponded to the time of ADAMTS13 testing results becoming available. Correlation between A-IPC and ADAMTS13 activity at presentation were also determined. Overall changes in PLT and A-IPC from daily pre-TPE CBCs in response to therapy were monitored. PLT and A-IPC curves were created using Excel 2013 (Microsoft Corp., Redmond, WA). Medical records both electronic as well as apheresis procedure logs were analyzed for adverse events. These were defined as events that required either pausing procedures with or without treatment of the patients and/or changes in vital signs.

ADAMTS13 activity was undetectable (< 5%) with mean inhibitor units (IU) of 5.7 (range 1.4–8) (Table 1). Six patients were female and two male; 4/8 (50%) of patients were African-American, 3/8 (37.5%) were Caucasian, and 1/8 was of mixed ethnicity. Mean age was 41 (range 17–63). Mean follow-up time was 28 days (range 10–45). Seven patients (88%) were obese (mean BMI 38.7 kg/m^2 [range 23.5–69.2]). All were Rh(D) positive, 4 were type O, 2 type A, and 2 type B. Patients had anemia (mean hematocrit 25.1% [range 20.4–32.7], normal 36–46%), elevated lactate-dehydrogenase (mean 1107 U/L [range 355–1838 U/L], normal 84–246 U/L), low haptoglobin (< 30 mg/dL, normal 30–200 mg/dL), thrombocytopenia (mean 13.5×10^9/L [range $5–29 \times 10^9$/L], normal $150-450 \times 10^9$/L), low A-IPC (mean 1.9×10^9/L [range $0.3–6.5 \times 10^9$/L] normal mean $7.1 \pm 0.5 \times 10^9$/L).

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Table 1
Demographics, laboratory data and therapeutic approach for cohort. All patients had ADAMTS13 activity < 5% at presentation with ADAMTS13 inhibitor as depicted. High inhibitor was arbitrarily defined as more than twice the level of that used to diagnose TTP (> 0.4 IU). 5/8 patients were previously hospitalized for TPE and all patients received additional long-term immunosuppression (LTIS) during current hospitalization. Data were appropriate indicated mean and range for cohort. F: Female; M: Male; C: Caucasian; AA: African-American; ATR: Allergic Transfusion Reaction; FNHTR: Febrile Non-hemolytic Transfusion Reaction; CE: Citrate Effect; BMI: Body Mass Index; Hapto: haptoglobin; TPE: Therapeutic plasma exchange.

Patient	Age	Gender	Ethnic background	PLT ($\times 10^9$/L)	A-IPC ($\times 10^9$/L)	ADAMTS13 inhibitor (U/L)	Number of TPE	LTIS	Day of LTIS initiation	ABO Rh (D)	Hct (%)	LDH (U/L)	Hapto (mg/dL)	Number of prior admissions	Adverse events
1	63	F	C	27	3.6	8	4	Cytoxan	4	O pos	32.7	355	< 30	4	None
2	51	F	AA	11	1.1	5.6	44	Rituximab	35	A pos	20.7	1838	< 8	1	ATR ×9
3	17	F	Mixed	7	1.7	8	34	Rituximab	27	B pos	20.4	1165	< 30	1	ATR ×4 CE X1
4	26	M	AA	9	0.5	1.4	13	Rituximab	15	A pos	24.4	1337	< 8	0	FNHTR ×1 CE ×1
5	51	F	C	10	0.7	8	19	Rituximab	14	B pos	24.4	858	< 8	4	None
6	44	F	AA	5	0.3	6.4	13	Cytoxan	5	O pos	24.8	797	< 30	0	None
7	27	F	C	10	0.7	2	5	Rituximab	4	O pos	21.1	1744	< 30	0	None
8	49	M	AA	29	6.6	6	9	Rituximab	7	O pos	31.9	763	< 30	3	ATR ×3
Mean (range)	41 (17–63)			13.5 (5–29)	1.9 (0.3–6.5)	5.7 (1.4–8)	17.6 (4–44)		13.9 (4–35)		25.1 (20.4–32.7)	1107 (355–1838)			

Patients were initiated on daily TPE and high dose prednisone; additional immunosuppression consisted of rituximab 375 mg/m^2 (6 patients) and cyclophosphamide 400 mg/m^2 (2 patients). 141 procedures were performed (mean 17 [range 4–44]). 5/8 patients had prior hospitalizations due to TTP. All patients had a 3-fold A-IPC increase (mean 11.1 × 10^9/L [range 2.2–25.3 × 10^9/L]) soon post-TPE initiation (mean 2.4 days [range 1–4 days]). A-IPC change preceded rapid PLT improvement (Fig. 1); however, PLT improvement was not accompanied by A-IPC return to baseline seen in TTP (negative feedback) without measurable inhibitor.

After patients achieved PLT (mean 217.6 × 10^9/L [range 200–294 × 10^9/L]) and A-IPC (mean 19.4 × 10^9/L [range 13–28.5 × 10^9/L]) they had concurrent decreases in both counts after a mean of 11.6 days (range 8–14 days), mean PLT was 65.4 × 10^9/L (range 14–176 × 10^9/L) and mean A-IPC 3.2 × 10^9/L (range 0.7–6.6 × 10^9/L). These decreases were of lesser severity as treatment progressed so that each was followed by modest increases in both parameters over time. Immunosuppression with either rituximab or cyclophosphamide therapy in conjunction with TPE (Table 1) was initiated after a mean of 14 days. Return of A-IPC to baseline occurred around day 25 and counts normalized after a mean of 45 days when divergent lines of A-IPC and PLT occurred without further PLT decreases. After TPE completion and PLT recovery, 4/8 patients remained with low ADAMTS13 activity and detectable inhibitor.

During TPE 4/8 patients had adverse events: one patient had nine allergic transfusion reactions, one had four allergic reactions and one reaction to citrate anticoagulation, a third patient had a febrile reaction and a citrate reaction, and the fourth patient had three allergic reactions (Table 1). Those patients who had allergic reactions to plasma had resolution with additional diphenhydramine and patient who had a febrile reaction to plasma improved with additional acetaminophen. Patients with allergic reactions completed their procedures after symptoms resolution. Patient with febrile reaction did not complete that procedure.

TTP treatment and diagnosis have improved since both TPE and ADAMTS13 testing began to be utilized. However, therapy responses can be affected when overlapping features leading to significant thrombocytopenia suggest a complex presentation [13,14]. Our data indicates that TTP patients with high ADAMTS13 inhibitor have A-IPC dynamics suggestive of count instability and potentially protracted response to therapy. These patients paradoxically experienced concurrent declines in A-IPC and PLTs while on therapy indicative of alternative mechanisms that uncouple the negative feedback characterizing disease recovery.

We previously reported that A-IPC is useful to identify patients with ADAMTS13 deficiency and gauge patients' response to TPE [9]. When co-existing mechanisms lead to immune dysregulation in the setting of TTP, A-IPC could aid in diagnosis and therapy response [10]. These consistent A-IPC dynamics of TTP patients in response to therapy lend support for its use in clinical trials [15]. Our cohort had count instability during their hospitalization and had rapid A-IPC improvements preceding PLT recovery; however, unlike our prior observations these were followed by sudden concurrent declines in both A-IPC and PLT while on daily therapy. If patients had achieved recovery, A-IPC would have returned to baseline. Therefore, absence of an A-IPC/PLT negative feedback indicative of recovery raises suspicions for protracted response. This suggests that discontinuation in the absence of negative feedback restoration may be premature.

A-IPC recovery occurred after a couple of procedures and was achieved prior to ADAMTS13 activity and inhibitor testing results became available. Interestingly, recurrent decreases in A-IPC and PLT occurred simultaneously during the first 21 days of treatment similar to what we have reported [10]. Possibly, other mechanisms may have led to the sudden but reversible decreases in A-IPC which did not predict PLT decreases. These mechanisms could lead to immature platelet production suppression and/or rapid clearance of these platelets.

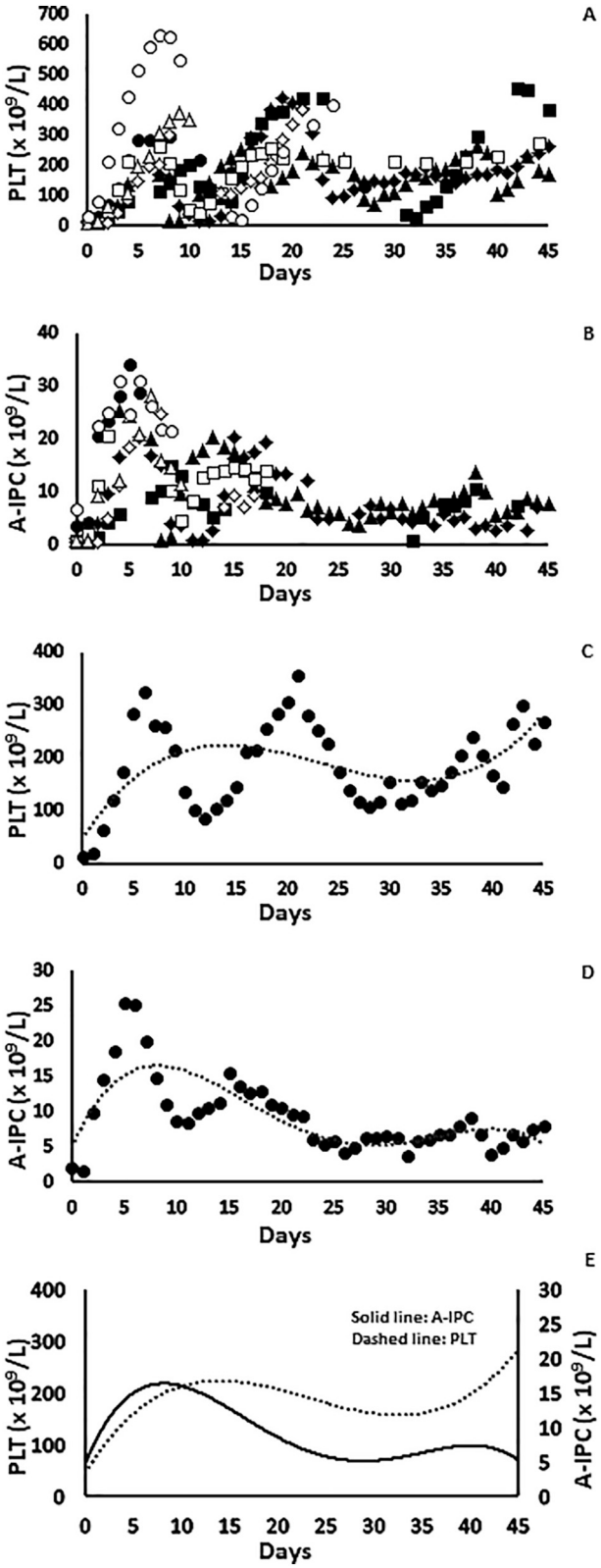

Fig. 1. Platelet count (PLT) and absolute immature platelet count (A-IPC) dynamics of cohort. Scatter plot of PLT (A) and A-IPC (B) for each day of treatment and follow-up. Each symbol is representative of a patient. Composite using mean for each treatment day with corresponding best-fit curve for PLT (C), and for A-IPC (D). Day 0 represents counts immediately prior to TPE initiation and each day thereafter represents day post-TPE initiation. Combined fig. (E) includes best-fit curves for PLT and A-IPC. Dashed line and solid line indicate PLT and A-IPC respectively. A reciprocal negative feedback is shown for PLT which when normalized leads to a corresponding A-IPC return to baseline. A-IPC recovery occurred earlier after treatment initiation compared to PLT.

New-onset TTP and relapsing TTP may represent dissimilar presentations leading to ADAMTS13 deficiency. In relapsing TTP antibodies to ADAMTS13 may have specificities different from those detected during new-onset since patients who relapse have antibody specificities different from those antibodies at initial presentation [16]. In TTP patients with active disease, complement mediators and anti-ADAMTS13 IgG have been described as being higher than patients who have recovered in the setting of higher cytokines concentration indicative of T cell-mediated immune dysregulation [17]. Potentially, plasma replacement could lead to immunomodulatory effects but this would affect all TTP patients since plasma is the replacement fluid used during TPE. Therefore, -at least in high-inhibitor patients- plasma exposure may not explain the observed suppression in production and/or removal of immature platelets. Instead, an inhibitory production event occurring during the first three weeks, such as *de novo* antibody formation and/or cytokine dysregulation which is exacerbated during therapy could be behind this consistent response to TPE.

Differences in A-IPC at presentation is likely disease-specific and reinforces that immune processes can lead to responses out of proportion with the thrombocytopenia [7], decreased as in TTP patients [9], or similar to normal range [8]. Therefore, A-IPC at presentation can not only be diagnostic but potentially increase clinical suspicion of a particular disease process.

Early use of immunosuppression could be helpful in patients with high inhibitors. Our results suggest that patients' A-IPC became stable once longer-acting immunosuppression was given. All patients received steroids but regardless of concurrent use with TPE count instability was not resolved. As reported in difficult to treat TTP patients, studies looking at early administration of agents such as rituximab indicate that patients recover their counts faster with fewer relapses [11,18]. Therefore, even though PLT are low and TPE should not be delayed, algorithms utilizing stronger immunosuppression earlier may be more successful, leading to count stability and sustained recovery in patients with high inhibitors.

In summary, A-IPC dynamics of patients with high anti-ADAMTS13 showed count instability during therapy and delayed A-IPC/PLT negative feedback restoration. Our results suggest that processes that either lead to suppression or increase removal/clearance of immature platelets may be at play. Recovery from disease only occurred once negative feedback is restored. A-IPC responses could be used to gauge if alteration to treatment and stronger immunosuppression should be used earlier in the presentation.

Declaration of Competing Interest

The authors report no conflicts with the submission of this manuscript.

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