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To the editor:

Platelet soluble CD40-ligand level is associated with transfusion adverse reactions in a mixed threshold-and-hit model

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Platelets are the principal source of soluble CD40-ligand (sCD40L) found in blood.[1] This biological response modifier has been reported to be a candidate mediator for acute reactions after platelet transfusions.[2] Serious adverse reactions (SARs) associated with excessive levels of sCD40L are febrile nonhemolytic transfusion reactions, transfusion-related acute lung injury (TRALI), and allergic reactions,[2-4] although there is not a consensus on the role of sCD40L in TRALI.[5,6] An elevated level of sCD40L in SAR-causing transfusions was found to be 1 element of a broader spectrum of biological response modifiers.[7-9] sCD40L was modeled for likeliness to be predictive of SARs compared with other mediators:[10] in the presence of elevated levels of sCD40L, elevated levels of MIP1α were associated with febrile nonhemolytic transfusion reactions, whereas low levels were associated with allergic type reactions.[7] In SARs, exacerbated sCD40L was attributed to platelet secretion in bags.[9] Although there is extensive polymorphism in genes that control both sCD40L isotype and production in donors,[11,12] no such polymorphisms proved statistically associated with SARs.[12] We evaluated whether elevated levels of infused sCD40L associate with SARs by following a large series of platelet concentrate (PC) transfusions.[7,8,10] Most patients who developed SARs received a high amount of sCD40L, but many patients who also received high amounts of sCD40L did not develop a SAR. Thus, only a subgroup of recipients manifested inflammation.

Single-donor apheresis (SDA) PCs and pooled platelet concentrates (PPCs) were produced in 1 regional setting of the French Blood Establishment). All PCs were leukoreduced to <10^6/bag and suspended in 35% native plasma/65% nutritive solution.[7,8,10] PCs from female donors were tested for anti-HLA. Platelets became outdated 5 days (d5) after collection. The study included 9206 successive PCs (issued to patients in the 2 university hospitals), of which 2850 were sampled at the time of delivery to the patient, allowing case control for the day of processing. A total of 140 SARs (grades 2-3; accountability 2-3)[9] was reported, for which samples were shipped back to the Blood Bank. The processing procedure and the incident/accident declaration strictly conformed to the national protocols.[8] Platelets incriminated in a SAR were immediately shipped back the Blood Bank for investigation. All samples obtained from PCs not associated with SAR (referred to as "no.AR") were used as controls. All samples were prepared as reported,[7,8,10] stored at −80°C, assayed for sCD40L by Luminex (HCYTOMAG-60K-06, Merck Millipore, Molsheim, France), and analyzed using Bioplex Software (Biorad, Marnes-la-Coquette, France). Statistical analyses consisted in paired *t* and nonparametric Mann-Whitney *U* tests. Analysis of variance, followed by the Kruskal-Wallis test with Dunn posttest, were performed to compare sCD40L concentrations during storage (GraphPad, La Jolla, CA). "Pathogenic" thresholds were calculated by

Table 1. Storage time of platelet components and adverse reactions

Days	No adverse reaction (n = 2710)*		Serious adverse reaction (n = 140)†	
	SDA PCs‡	Pooled PCs§	SDA PCs‡	Pooled PCs§
1-2‖	162	269	6	13
2-3	402	395	15	12
3-4	491	279	25	20
4-5	377	335	29	20
Total (n = 2850)	1432	1278	75	65

*No adverse reaction reported.

†Serious adverse reaction reported.

‡Single donor, apheresis platelet component.

§Whole blood buffy coat–derived blood component, pools of 5 ABO-RH:1 matched units, constituting 1 pooled platelet component.

‖Indicates that more than 24 hours have elapsed since the computer integrated timeframe of blood donation and less than 48 hours (maximum, 47 hours).

a "decision stump," which is a binary test focusing on an "attribute" (ie, sCD40L) and allowed minimizing the "entropy/uncertainty" (group impurity [ie, the risk of having negative data mixed with positive data in the same group, such as the SAR and no.AR groups]).[13]

Of 9206 consecutive PCs enrolled in this survey, 2850 were successfully processed, making this study highly powered (not all PCs could be sampled because of the constraints of normal practice). From these 2850 PCs (1507 SDA PCs and 1343 PPCs), for which biological material was available both at the time of labeling (d0-d1) and issuance (d1-d5), 2710 were not associated with a SAR (no.AR; 1432 SDA PCs and 1278 PPCs) and 140 were associated with a SAR (75 SDA PCs and 65 PPCs) (Table 1). Figure 1 shows sCD40L levels in SDA PCs (Figure 1A,C,E) and PPCs (Figure 1B,D,F) and the calculated thresholds (6424.01 and 6182.68 pg/mL, respectively) for each delivery day (d0-d5) that led to no.AR or to a SAR. Most no.AR values fell below the "pathogenic" sCD40L threshold calculated from a decision stump: 82% and 18% were < and ≥6424.005 pg/mL sCD40L, respectively, for SDA PCs, and 54% and 46% were < and ≥6182.68 pg/mL sCD40L, respectively, for PPCs (Figure 1C-D). Conversely, most (60% SDA PCs and 82% PPCs) SAR values were ≥6424.005 or ≥6182.28 pg/mL sCD40L, respectively (Figure 1E-F). The percentage of SARs in the SDA and PPC patient groups was similar (0.4977% vs 0.484%). However, the frequency of SARs increased with time in storage, particularly in the SDA PC group, in accordance with previous findings.[14]

Only patients who received only 1 platelet transfusion with the exclusion of other components were enrolled in the present survey to ensure that the considered SARs were indeed linked to the platelet transfusion; this does not preclude that other blood components were not transfused before the platelet component having caused the SAR. When that was the case, however, the last transfused blood component was given at least 16 hours before (16 hours being considered the timeframe to rely a SAR to a given blood component, according to the majority of published consensuses). A limit of this study is that it strictly depends on reporting. Many patients receiving PC transfusions received immunosuppressive drugs, such as corticosteroids, which may have masked symptoms[15] and caused underreporting. The aim of the methods used in this study to minimize entropy/uncertainty was to restore possibly altered data.[16] The influence of irradiation on sCD40L secretion[17] could not be assessed. Although most PCs were irradiated, samples were generally taken immediately before irradiation for logistical reasons. However, γ-irradiation does not augment platelet secretion of biological response modifiers, contrary to ultraviolet C.[18]

The present study did not aim to confirm previous data, but to provide new information on the link between sCD40L and SARs, namely to explore whether high levels of sCD40L are consistently associated with SARs, or not, in transfused patients. It has also been shown that the storage duration of PCs is associated with the secretion of sCD40L[19-21] and the occurrence of sCD40L-associated SAR.[12] Thus, we sought to further examine the relationship of sCD40L values and SARs, considering platelet concentrate storage time. We plotted the sCD40L levels in supernatants of the PCs associated with the 140 reported SARs against controls after various times of storage. The results are particularly suggestive of an "all or nothing" threshold phenomenon already proposed in case studies,[10] suggestive of the requirement for a second trigger in addition to higher sCD40L levels. Such a trigger is yet to be identified, but would fit well both the "2-hit" and "threshold" models of TRALI[22] and various clinical conditions.

We cannot rule out genetic causality, but in a previous study, we were unable to show an association between occurrences of SARs and genetic variants of *CD40LG*. We also cannot rule out the possibility that other genes are implicated, such as those regulating CD40 and CD40L pathophysiology.[11,12] Further genetic analyses of susceptibility may identify patients who are the most exposed to such a risk. This study clearly showed that sCD40L levels are not fully predictive of SARs, but leaves open the possibility of the comorbidities of the recipient, genetic susceptibility (high-affinity binding of sCD40L by off-target receptors), a causal disease condition, or all 3. Current means to reduce the risk of introducing excess sCD40L to transfusion recipients is either to use fresh (≤3 day old) PCs (but susceptible patients may nevertheless develop SARs even with this safety measure) or to eventually wash platelet components.[23-25]

Considering that the storage lesion side product sCD40L is pathogenic, at least in a non-negligible of susceptible recipients, several options are opened. The platelet-washing procedure is not only time-consuming but also does not prevent platelets from continuing to secrete biological response modifiers, prone to accumulate during storage. In a number of diseases, recently developed biologicals to either CD40L or CD40L were successfully used; however, these drugs may interfere with patients' own platelets. Some studies have succeeded in removing 80% to 90% of sCD40L in PCs using a column of adsorptive cellulose beads: these processes usually decrease the recovery of platelets after adsorption but do not alter platelet function and may represent the best option to reduce severe nonhemolytic transfusion reaction if needed. It should be proposed that a batch system be engineered to absorb not only sCD40L but also inflammatory platelet biological response modifiers while preserving the functionality and quality of PCs, optimally being washable/reusable to make the process affordable and cost-effective.

Contribution: O.G. undertook the study hypothesis and wrote the manuscript; F.C. designed the protocol, trained personnel in blood banks, and contributed to the writing of the manuscript; C.S. collected samples, did the experiments and statistical analyses, and cowrote the manuscript; E.F. performed all stump decision and big data analyses; and S.L. and H.H.-C. participated in all steps of the process and reviewed the manuscript.

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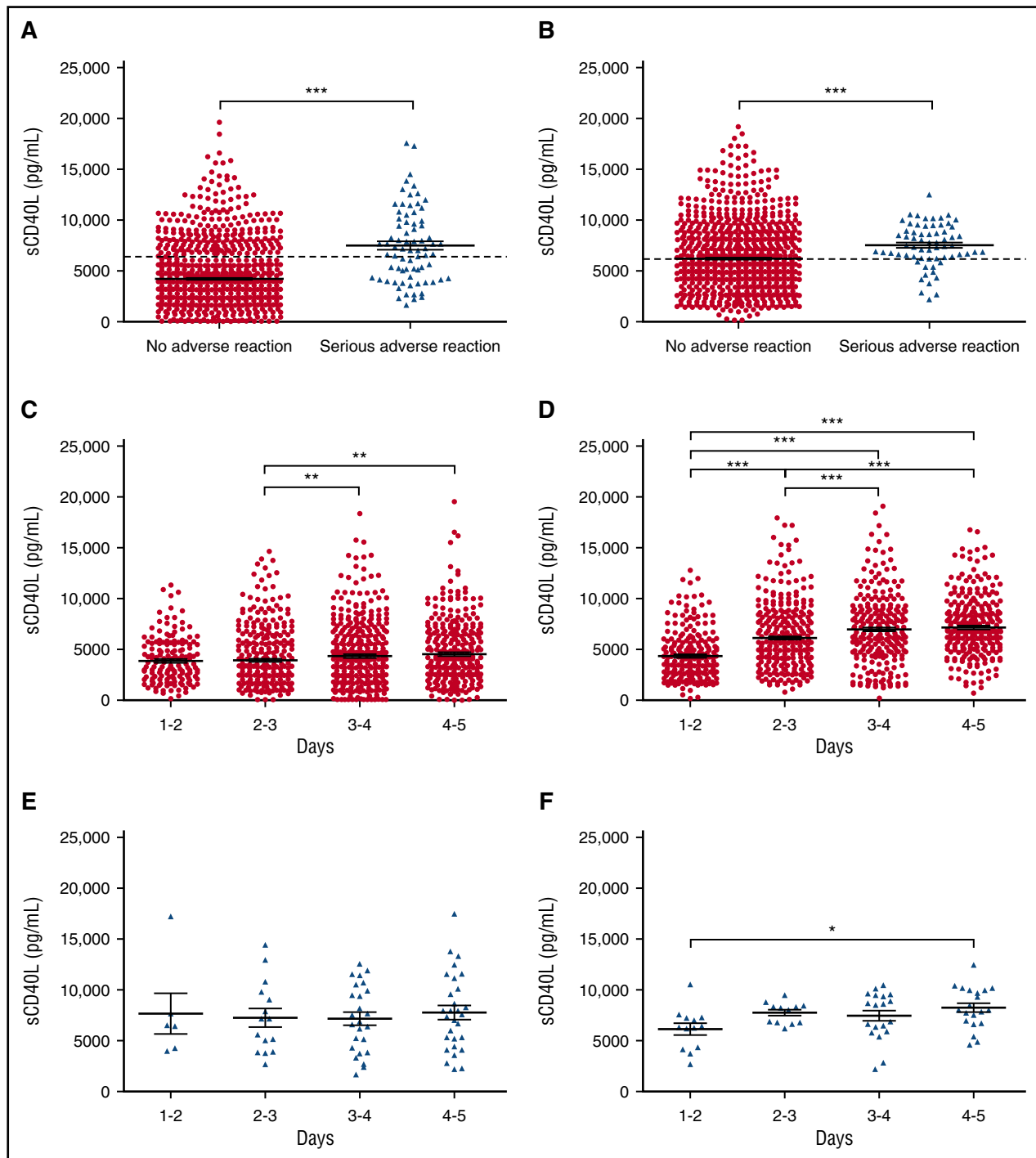
Figure 1. Increase of sCD40L in supernatants. Increase during the storage of SDA platelet components (A,C,E) and pooled platelet component (B,D,F). Day 5 represents the last day of storage for which SDA platelet components and pooled platelet component can be used for transfusion in our study. The production of sCD40L was quantified by Luminex technology. Values shown are deducted from background levels. Data (scatter plot and mean; n as indicated in Table 1) are expressed in picograms per milliliter. Group comparisons were performed by 1-way analysis of the variance followed by the Kruskal-Wallis test with Dunn posttest. The 2-tailed t test and Mann-Whitney test were used to compare 2 groups ($*P < .05$; $**P < .01$; $***P < .001$).

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To the editor:

Hematologic relapse in AL amyloidosis after high-dose melphalan and stem cell transplantation

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Light-chain (AL) amyloidosis is a rare disease in which an underlying clonal plasma cell population generates aberrant immunoglobulin light chains that misfold and form amyloid fibrils, which are deposited in extracellular tissues and organs, resulting in impairment of vital organ function.[1,2] High-dose melphalan and autologous stem cell transplantation (HDM/SCT) can produce both hematologic and clinical remissions and extend survival in selected patients with AL amyloidosis.[3-5] However, there are limited data documenting outcomes for those who experience hematologic relapse after an initial hematologic complete response (CR) to HDM/SCT. We report on patients with AL amyloidosis treated with HDM/SCT to review long-term relapse and survival data and to help guide the intricate management and follow-up of this unique patient population.

Our group previously reported on 629 patients with AL amyloidosis who underwent HDM/SCT between 1994 and 2014. These patients were enrolled in several successive institutional review board–approved protocols with inclusion criteria as previously reported.[3] Hematologic response was assessed using international consensus criteria,[6,7] and 40.3% achieved a CR at 6 to 12 months following HDM/SCT. The CR rate was 34.8% by intention-to-treat. At the time of that report, hematologic relapse had occurred in 40 patients (18.2%), having previously achieved CR with a median time to relapse of 3.97 years (range, 1.89-12.45). The median overall survival (OS) for patients following hematologic relapse was 4.3 years. A retrospective review of 410 patients who underwent HDM/SCT for AL amyloidosis from 1996 to 2009 at Mayo Clinic revealed hematologic relapse or progression in 146 patients (36%) with a median time to relapse/progression of 1.97 years.[8] The median OS in this patient group after relapse or progression was 4.31 years, and the most common treatment regimen pursued after progression included lenalidomide or thalidomide. We note however that only 38 of the patients (26%) investigated with relapse/progression had achieved a hematologic CR post-SCT. Others have reported an event-free survival of ~4 years in patients undergoing SCT for AL amyloidosis independent of hematologic response and superior event-free survival in those individuals achieving CR at 1 year posttransplant.[5] Therefore, there remains a scarcity of information available regarding outcomes for relapsed patients after HDM/SCT in AL amyloidosis.