Letters to the Editor

Vox Sanguinis

Factor VII and Activated Factor VII Content of Prothrombin Complex Concentrates

H. Fiedler
Münster, Germany

Hellstern et al. [1] compared the activated factor VII (FVIIa) content of a prothrombin complex concentrate (PCC) that had been involved in three fatal thromboembolic complications with that of another licensed PCC whose complication rate the authors did not impart. They found higher FVIIa values in the first-mentioned preparation. From this observation, the authors concluded that higher FVIIa potencies may contribute to the thrombogenic potential of PCC. Such an inference seems to me to be at variance with the well-established theory that FVIIa in the absence of tissue factor (TF) is not thrombogenic at all [e.g. 2], while in the presence of TF nonactivated FVII is converted into FVIIa within a few seconds [3]. This theoretical consideration is not ruled out by the result of the above-mentioned comparison.

Occasionally, cases of acquired prothrombin complex factor deficiency are due to (or accompanied with) a more or less compensated intravascular activation of the clotting cascade, e.g. by TF exposed to the bloodstream. Under such circumstances, any replenishment of the intravascular FVII pool, even with nonactivated FVII, can lead to the decompensation of the local or disseminated intravascular coagulation process, followed in the worst case by local thrombosis and/or disseminated intravascular coagulation. Therefore, in order to avoid a possible selection bias, the choice of PCC preparations for assessing putative markers of irregular (i.e. TF-independent) thrombogenicity should principally be confined to preparations that were involved in thrombogenic events in patients without preexisting elevations of activation markers. The authors did not provide information on how they circumvented this kind of a possible selection bias. Consequently, the utmost inference that can be drawn from the article is that it remains to be determined whether a high FVIIa content of a PCC can make it irregularly thrombogenic.

References


Dr. Harald Fiedler
Stellmacherweg 5
D–48161 Münster (Germany)

Authors’ Reply

P. Hellstern, H. Beeck
Institut für Hämostaseologie und Transfusionsmedizin
Klinikum Ludwigshafen GmbH, Ludwigshafen, Germany

Dr. Fiedler claims that it makes no difference whether native factor VII (FVII) or activated factor VII (FVIIa) is infused to patients. It is true that FVIIa by itself has only little proteolytic activity [1]. However, there are several findings indicating that even low levels of FVIIa, but not FVII may serve as a priming function for triggering the clotting cascade [2]. It has been shown that FVIIa at picomolar concentrations can effectively activate FVII bound to tissue factor (TF) [3]. FVIIa itself rapidly forms complexes with TF and phospholipids that are able to activate factors IX and X efficiently. Trace amounts of TF are detectable in the plasma of normal individuals, and substantial amounts of TF exposed to the bloodstream are found in arterial and venous thromboembolism and in disseminated intravascular coagulation (DIC) [4].

It is an important fact that, in contrast to recombinant FVIIa and activated prothrombin complex concentrates (PCC), nonactivated PCC are commonly not administered to patients with hereditary coagulation factor deficiencies. With the rare exception of hereditary deficiencies of factors II, VII, and X, PCC are used for replacement of vitamin K-dependent clotting factors and inhibitors in liver disease and phenprocoumon or warfarin overdose. Patients suffering from liver disease are prone to DIC. Rapid reversal of oral anticoagulation is accompanied by the risk of recurrent thromboembolism as a consequence of a hypercoagulable state found in most of these patients. Hence, the suggestion of Dr. Fiedler to restrict observations dealing with thrombogenicity to patients without activated hemostasis cannot be accepted, since this would lead to a dangerous underestimation of the thrombogenic potential of PCC.

In spite of the fact that exact features and mechanisms determining thrombogenicity of PCC are presently unknown, it is well conceivable that DIC or hypercoagulability may be deteriorated by PCC containing high activities of activated clotting factors including FVIIa [5]. However, the PCC involved in thromboembolic complications in Germany had further features which are thought to be associated with thrombogenicity. This preparation contained not only high FVIIa potencies, but was also substantially overloaded with FII, while being free of antithrombin and protein S activity [6, 7].

In a previous comparative study, the preparation in question had already attracted attention by causing severe cardiovascular complications in rabbits [8]. The lots involved in these complications were also overloaded with prothrombin and free of antithrombin. Meanwhile, one life-threatening and seven fatal complications associated with the use of this PCC have been observed in Germany [6, 7, Köhler et al.: unpubl. data]. This arouses strong suspicion of a causal relationship, particularly since no fatal complications have been reported in Germany after the application of other licensed PCC during the last 10 years.
References


Peter Hellstern, Hannelore Beeck
Institut für Hämostaseologie und Transfusionsmedizin
Klinikum Ludwigshafen GmbH
Bremerstrasse 79
D–67063 Ludwigshafen (Germany)
Tel. +49 621 5033500, Fax +49 621 5033520
E-Mail Peter.Hellstern@msn.com

Vox Sang 1998;75:73

Hemoglobin Determination in Blood Donors
Tomislav Vuk, Dorotea Šarlija, Melita Balija, Damir Grgičević
Croatian Institute of Transfusion Medicine, Zagreb, Croatia

There have been a number of controversies about the accuracy, precision, sensitivity and specificity of various Hb assays, especially regarding differences between venous and capillary assays. This study was conducted to examine the relationship between Hb concentrations obtained by capillary and venous methods and between venous Hb concentrations before and after donation. 50 prospective blood donors (48 males and 2 females) were included in the study. EDTA-anticoagulated venous blood samples were obtained immediately before and immediately after 450-ml whole-blood donations. Venous Hb concentrations were measured on an automated counter (model MD 18 Coulter Electronics). The capillary Hb concentrations were determined on fingerprick blood samples obtained immediately before whole blood donation using a HemoCue B-hemoglobin photometer. The mean predonation Hb value determined was 157.5 ± 13.3 (range 135–195 g/l) using HemoCue B (capillary blood), and 145.7 ± 8.2 (range 126–164 g/l) using Coulter MB18 (venous blood). The mean Hb value of the post-donation venous sample was 139.9 ± 8.7 (range 119–158 g/l). The regression equation for the results obtained with HemoCue for capillary blood (y) versus those obtained with Coulter MD-18 (x) for EDTA venous blood was y = 0.37x + 87.1. The coefficient of correlation was 0.60. The differences between predonation capillary and venous Hb values using analysis of variance were statistically significant (p<0.001). These differences must be taken into consideration in determining the minimal acceptable Hb thresholds for the capillary assays. Because established donor acceptance policies are based on venous Hb standards, the same standards may not be appropriate for capillary assays. A statistically significant difference was found between venous Hb concentrations before and after donation (p<0.001) with the regression equation y = 1.0x – 7.6 and the correlation coefficient 0.95. In the paper by Boulton et al. [1] it was shown that after donation Hb values are consistently lower by an average of 3.5 g/l, but no statistical significance was found. It has been suggested that influx of extravascular fluid during donation, combined with recumbency, might lower the venous hemoglobin concentration. In our study, values below the threshold were found in 1 donor when Hb concentrations were measured before donation, and in 11 donors when venous samples were measured after donation. For this reason, after donation Hb values may not truly represent the state before donation.

Reference


Tomislav Vuk, MD
Croatian Institute of Transfusion Medicine
Petrova 3, 10000 Zagreb (Croatia)
Tel. +385 1 46 00 333, Fax +385 1 44 56 10
Determination of Glycocalicin in Platelet Concentrate Supernatants Stored in a Synthetic Medium (Seto Solution)

Shinji Kunishima, Tetsuo Shimizu, Shinichi Kora, Tadashi Kamiya, Kazuo Ozawa

Japanese Red Cross Aichi Blood Center, Seto, and R&D Center, Terumo Corporation, Nakai-machi, Kanagawa, Japan

Platelet concentrates (PC) for transfusion are routinely stored in autologous plasma. It has been suggested that replacing plasma with platelet additive solution should diminish the risk of allergic reactions to transfused plasma proteins [1]. We and others have developed synthetic media for platelet storage [2–5]. The Seto additive solution contains acetate, bicarbonate and glucose with a physiological pH [3–5]. The quality of platelets stored in plasma and in Seto solution has been compared in detail, with no differences found between the two media [3–5].

Glycocalicin is a soluble proteolytic fragment of the α subunit of glycoprotein (GP) Ib, the platelet receptor for von Willebrand factor [6]. Over the past 20 years, studies have shown that platelet membrane GPs, including GPIb, are susceptible to degradation during in vitro storage, suggesting that loss of glycocalicin from the platelet membrane might induce a deterioration of platelet function. The loss of GPIb from platelets during storage may in part be attributable to platelet activation and/or platelet vesiculation. The mechanism of GPIb loss during storage at 22°C is not currently understood; however, cold storage of platelets causes increased membrane vesiculation and accelerated loss of GPIb. Since all these previous studies have been performed on platelets stored in plasma, no information concerning changes in platelet GPs during PC storage in synthetic medium is available [7]. Using glycocalicin as a parameter, we examined whether replacement of plasma with Seto solution affects platelet membrane damage during storage.

PCs obtained with informed consent from ten normal volunteers were prepared by apheresis with CS-3000 plus® and Amicus® separators (Baxter, Deerfield, Ill.). PCs were either routinely resuspended in autologous plasma (plasma PC) or in Seto solution (Seto PC), with removal of approximately 85% of plasma at the final resuspension step in the apheresis process [3]. Both types of PC were prepared from the same donors and stored at 22°C under constant agitation for 6 days. Immediately after preparation (day 0) and after storage for 3 and 6 days, aliquots were withdrawn, centrifuged and the supernatants were collected. Glycocalicin concentration in the supernatant was determined by an enzyme-linked immunosorbent assay (ELISA) [8, 9]. During storage, glycocalicin concentration in the supernatants of plasma PC progressively increased from 1.76±0.18 µg/ml (mean ± SD; day 0) to 4.30±0.28 µg/ml (day 6); the normal plasma glycocalicin concentration is 1.40±0.25 µg/ml). Similar results were obtained with Seto PC supernatants (from 0.53±0.05 µg/ml at day 0 to 2.75±0.17 µg/ml at day 6; fig. 1). Glycocalicin concentrations increased in parallel during storage and were indistinguishable from those in plasma PC. Ristocetin-induced platelet aggregation also decreased slightly during storage in both media.

Although the precise reasons why glycocalicin concentration increases in PC supernatants remain to be determined, replacement of plasma with synthetic media did not adversely affect glycocalicin release or ristocetin-induced aggregation during storage. We have found that platelets stored in Seto solution for 5 days had a normal discoid shape; no fragmented or ballooned platelets were observed. The surface expression of GPIb, as estimated by flow cytometry, was not decreased as compared with that of plasma PC [3]. Taken together, as measured by loss of GPIb from platelet membranes, Seto solution is no worse than plasma for platelet storage. Determination of glycocalicin concentration should be useful in assessing methods for improving platelet preparation and storage conditions.

Fig. 1. Changes over time in glycocalicin concentrations in supernatants and ristocetin-induced platelet aggregation of PC stored in plasma and Seto solution
References


4 Shimizu T, Murphy S: Roles of acetate and phosphate in the successful storage of platelet concentrates prepared with an acetate-containing additive solution. Transfusion 1993;33:304–310.


Shinji Kunishima
Japanese Red Cross Aichi Blood Center
539-3 Minamiyamaguchi
Seto 489 (Japan)
Tel. +81 561 854292, Fax +81 561 843912
E-Mail kunisima@fujita-hu.ac.jp.

Membrane Adsorptive Properties of a New Polyurethane Leukocyte Reduction Filter in Comparison with Those of a Negatively Charged Polyester Filter

Aichi Center, Japanese Red Cross Blood Transfusion Service, Seto, Japan

Urticarial/anaphylactic reactions commonly occur after platelet transfusions, but the mechanisms involved are not well understood. Removal of anaphylatoxins by the negatively charged filter could be one method for reducing the incidence of those reactions. We have previously shown anaphylatoxin-scavenging abilities of negatively charged polyester leukocyte reduction filters, in which around 90% of anaphylatoxins of C3a and C4a were adsorbed with filtration of stored platelet concentrates (PCs) [1]. Since these anaphylatoxins are highly basic proteins and the same filter also adsorbed platelet factor 4 and β-thromboglobulin with high pI, we suggested a strong ionic interaction between the positively charged proteins and the negatively charged filter membranes [1]. However, the filter has the disadvantage of generating bradykinin (BK) [2], which causes hypotensive reactions in patients with decreased angiotensin-converting enzyme activity [3].

We here determined the membrane polarity of a new polyurethane filter as well as adsorption properties of some basic proteins in PCs and the generation of BK with a new filter.

The 3-day-old apheresis PCs were equally divided into two bags. Then paired PCs were each filtered through polyurethane Immugard III-PL (Terumo) and polyester PXL8H (Pall) filters at a flow rate of 5–7 ml/min. The C3a/C3a des Arg, C4a/C4a des Arg, and IL-8 levels were determined with commercially available diagnostic RIA and ELISA kits. To determine BK levels with ELISA kits (Dainippon Seiyaku), filtrates were collected in 1-ml tubes each. The zeta-potential of the polyurethane filter was measured by a screening potential method using a ZP-10B (Shimazu).

The zeta-potential of the new polyurethane filter is ~1.6 mV, indicating almost neutral surfaces. The filtration with the polyurethane filter had no effects on the C3a, C4a, IL-8, and BK levels (table 1). While significant amounts of C3a, C4a, and IL-8 were adsorbed in eluates with negatively charged filtration (table 1), BK was also dramatically generated in eluates.

This study showed that the new polyurethane leukocyte reduction filter with a neutral filter membrane neither adsorbs anaphylatoxins or IL-8 nor causes BK generation. The present data together with the recent report by Geiger et al. [4] support the previous conclusion that the adsorption properties by the negatively charged polyester filter are strongly related to ionic interactions between the negatively charged filter membranes and basic proteins.
Table 1. C3a, C4a, and IL-8 levels and generation of BK in eluates following filtration of 3-day-old apheresis PCs with neutral and negatively charged leukocyte reduction filters

<table>
<thead>
<tr>
<th></th>
<th>Neutral filter</th>
<th>Negatively charged filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3a ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before filtration</td>
<td>1.418±0.567</td>
<td>1.423±0.484</td>
</tr>
<tr>
<td>Half</td>
<td>1.532±0.649</td>
<td>2.46±0.127*</td>
</tr>
<tr>
<td>End</td>
<td>1.549±0.640</td>
<td>2.94±0.102*</td>
</tr>
<tr>
<td>C4a ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before filtration</td>
<td>1.944±0.338</td>
<td>1.804±0.366</td>
</tr>
<tr>
<td>Half</td>
<td>2.048±0.330</td>
<td>5.6±0.58*</td>
</tr>
<tr>
<td>End</td>
<td>2.250±0.188</td>
<td>25.1±0.335*</td>
</tr>
<tr>
<td>IL-8 pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before filtration</td>
<td>18.2±5</td>
<td>18.3±3.7</td>
</tr>
<tr>
<td>Half</td>
<td>16.7±3.6</td>
<td>8.3±3.1*</td>
</tr>
<tr>
<td>End</td>
<td>16.2±4.4</td>
<td>12.7±2.2*</td>
</tr>
<tr>
<td>BK ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before filtration</td>
<td>0.07±0.05</td>
<td>0.05±0.05</td>
</tr>
<tr>
<td>25 ml filtered</td>
<td>0.06±0.06</td>
<td>1.22±1.45*</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD of 5 preparations. *p<0.05 vs. the corresponding value before filtration (Student’s t test). Half = Filtrates were collected into a collection bag and filtrate samples were obtained from a collection bag to which a half volume of PCs was filtered; End = the filtrate sample was obtained from a collection bag at the end of filtration. Samples for BK determination were collected from a bag obtained before filtration and in eluates in which 25 ml of PCs had been filtered. Previous studies indicated peak responses of BK generation, when 25 ml of PCs had been filtered with the negatively charged filter.

References

Tetsuo Shimizu, PhD
Blood Transfusion Information Section
Aichi Center, Japanese Red Cross Blood Transfusion Service
539-3, Minami-Yamaguchi, Seto 489-8555 (Japan)
Fax +81 561 86 0176
Does HCV Screening of Blood Donors Affect Transmission of Hepatitis G Virus in Dialysed Patients?


Service d’Immuno-Hématologie, Service de Gastro-Entérologie, Service de Dialyse rénale et Service de Virologie fondamentale, Centre Hospitalier Universitaire de Liège, Sart Tilman, Liège, Belgique

GBVC, or hepatitis G virus (HGV), is a recently identified virus, transmissible by blood, which has been reported to be associated with HCV in around 20% of cases [1, 2]. Since screening for HCV antibodies in blood donors became mandatory in Belgium in July 1990, it appeared worthwhile to investigate a possible effect of HCV donor screening on the transmission of HGV in patients undergoing hemodialysis. The patient cohort studied included 82 dialyzed patients (51 men, 31 women) aged 18–81 on dialysis for periods between 2 months and 28 years. In addition to their transfusion status, we also analysed known risk factors such as dialysis and kidney transplantation carried out prior to and after July 1990 for parenteral transmission of HCV and HGV. The HBV, HCV, and HGV status of patients was determined by the detection of HBsAg (Auszyme Monoclonal and HBsAg Neutralization Assay, Abbott), anti-HCV (HCV EIA 3.0 and HCV Supplemental Assay, Abbott), and HGV RNA by RT-PCR using primers (i.e. G8-G11) derived from the NS3 genomic region of HGV [3].

The overall prevalence of viral markers in our patient cohort was 11% (9/82) for anti-HCV, 15.9% (13/82) for HGV RNA and 3.7% (3/82) for HBsAg. HGV RNA was more often found alone (9/13), while in the coinfected it was associated with either HBsAg (2 patients) or anti-HCV (2 patients). HGV RNA was never found associated with concurrent HBV and HCV infection. Interestingly, liver enzymes (ALT) in 10 of 13 HGV RNA-positive patients were normal. Of the 3 patients with elevated ALT levels, 1 was coinfected with HBV (HBeAg-positive), the second with HCV, and the third had fatty liver infiltration associated with insulin-dependent diabetes.

As far as the prevalence of HGV infection is concerned, there was no statistically significant difference between patients who were dialyzed, transfused or transplanted prior to or after July 1990. It is worth noting that the 13.3% (4/30) prevalence of HGV RNA among patients transfused after 1990 was lower than the 20% prevalence (8/40) among those transfused prior to 1990. However, this difference did not reach statistical significance. As expected, implementation of anti-HCV screening in 1990 was strongly associated with a dramatic reduction in transmission of HCV in dialyzed and transfused patients. In fact, the seroprevalence of HCV fell from 20% (8/40) prior to 1990 to 3.3% (1/30) after 1990 (p<0.05). In all but 1 polytransfused patient (see patient (a) in table 1), HCV infection could be traced to transfusions received prior to 1990.

We conclude that HCV screening of blood donors proved to be efficient in preventing blood transfusion-transmitted HCV in our cohort of dialyzed patients without a concomitant significant effect on the transmission of HGV.

References

1 Barbara J: Does GB virus C (hepatitis G virus) threaten the safety of our blood supply? Transfus Med 1997;7:75–76.

Christiane Gérard, PhD
Immuno-Hématologie and Transfusion
Centre Hospitalier Universitaire de Liège
Bât. B35, niv. 0
B–4000 Sart Tilman, Liège (Belgium)
Tel. +32 4 366 75 39, Fax +32 4 366 75 47

Table 1. Prevalence of HGV and HCV in patients who began to be dialyzed, transfused or transplanted prior to, or after July 1990

<table>
<thead>
<tr>
<th>Medical event</th>
<th>Before/after 1990</th>
<th>HGV positive</th>
<th>HCV positive</th>
<th>χ2: p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>negative</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First dialysis</td>
<td>before</td>
<td>6</td>
<td>22</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>after</td>
<td>7</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Transfusion</td>
<td>before</td>
<td>8</td>
<td>32</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>after</td>
<td>4</td>
<td>26</td>
<td>1*</td>
</tr>
<tr>
<td>Transplantation</td>
<td>before</td>
<td>4</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>after</td>
<td>2</td>
<td>5</td>
<td>1*</td>
</tr>
</tbody>
</table>

* Between 1995 and 1997, this polytransfused patient received 75 packed RBC, 150 plasma and 73 platelet units.

b Patient was transfused before July 1990.
Asymptomatic Type II Mixed Cryoglobulinemia with a Very High Cryocrit Level Secondary to HCV Infection

Paolo Perseghin, Pierpaolo Parravicini, Giovanna Farina

Servizio Trasfusionale and Divisione di Medicina Interna, Azienda Ospedaliera 'E. Morelli', Sondalo, Italy

A 55-year-old woman was referred in April 1997 for elevated liver enzymes detected during routine preoperative screening for minor surgery. She was asymptomatic and clinical examination was negative except for mild purpuric lesions on her lower limbs. She had received multiple blood transfusions 20 years earlier during surgery for spinal fusion. Her ALT levels were 367 U/l and AST 198 U/l (normal values <50 U/l). Serology was negative for HBV and positive for anti-HCV both with third generation ELISA and RIBA. An HCV quantitative assay (Amplior, Roche, Italy) and genotyping (INNO-Lipa, Innogenetics, Belgium) were performed following the manufacturer’s instructions, showing elevated viremia ($144 \times 10^3$/ml copies of HCV-RNA), while genotyping revealed a $2a,c$ strain (Simmonds). Moreover, monoclonal IgMk with strong RF activity ($>40,000$ IU/ml) with a polyclonal IgG were detected using standard methods [1]. Anti-HCV testing was performed on cryoprecipitate, using ELISA and RIBA [2]. Small samples were diluted with a fixed amount of warm (37°C) saline to final dilutions ranging from 1/10 to 1/10,000. All were strongly positive by ELISA (OD $>2.200$ = out of range) as well as by RIBA. All bands showed a 4+ positivity until 1/1,000 dilution, then a 2 to 3+ positivity at 1/10,000. Intrinsic control bands and the SOD band were negative. HCV-RNA quantitation was not performed on cryoprecipitate.

C3 and C4 were in the normal range. Cryocrit was extremely high (76%); plasma and whole blood viscosity were 1.85 mPa·s (normal values $1.03 \pm 0.09$) and 3.94 mPa·s (normal values $3 \pm 0.39$), respectively. Type II mixed cryoglobulinemia was diagnosed. Liver biopsy showed features of chronic active hepatitis (HAI score 10, stage 2). The patient refused biopsy of her skin lesions. In order to avoid the risk of cardiovascular events related to hyper viscosity [3], the patient underwent two plasma exchange procedures on alternate days (one plasma volume each with saline and 5% human albumin), resulting in a drop of cryocrit from 76 to 13.5%. Thereafter, r-αIFN2a 3 MU/daily sc was started. After 4-month follow-up, ALT and AST normalized (fig. 1), viremia was no longer detectable but the cryocrit was still elevated. At present, the patient complains only of mild arthromyalgias, probably a side effect of the IFN therapy. This case has two unusual features. (1) The woman lives an active life in a Northern Italian alpine valley, where the winter temperature usually falls below $-15^\circ$C. It is very unusual that such a high cryocrit does not lead to evident clinical manifestations, even if it is well known that cryoglobulin-related symptoms are not strictly related to the pathologic protein thermal range and plasma concentration [4]. (2) Moreover, the persistence of high cryocrit levels after a 4-month course of IFN therapy, during which liver enzymes normalized and viremia disappeared, suggests that this finding might be due to the possible presence of HCV in infected B lymphocyte clones [1, 5, 6], which could in turn trigger the long-lasting production of the pathologic antibody. In conclusion, serial cryocrit determinations should be performed to identify whether a complete or partial response is achieved [7], but costly and ineffective IFN therapy should be withdrawn.

Fig. 1. ALT (●) and cryocrit (□) levels at a 4-month follow-up. Controls performed at biweekly intervals. Between controls 1 and 2, two plasma exchange procedures were performed.
Letters to the Editor

References


P. Perseghin, MD
Servizio Trasfusionale e di Immunematologia
Ospedale San Gerardo de’ Tintori
Via Solferrino 16
I-20052 Monza (Italy)
Fax +39 039 2301380

Vox Sang 1998;75:79–80

Relationship between Antibodies Dependent on Calcium Chelators and the H Antigen

D. Janvier *, M. Reviron **, J. Maury *
Sites transfusionnels
* Saint-Louis,
** Trousseau, et
* Tenon,
Etablissement de Transfusion Sanguine
de l’Assistance Publique-Hôpitaux de Paris, France

A case of citrate-dependent autoanti-H, responsible for an ABO typing problem, was recently described by Joshi [1]. This paper questioned again why some autoantibodies react only in the presence of calcium chelators: the role of carboxyl groups [2] or the inhibitory effect of ionized calcium [3] was invoked. Over a period of 8 years, we encountered four ABO typing discrepancies due to antibodies reacting in the presence of calcium chelators. Remarkably, all four antibodies were specific for the H antigen or a related structure. They behaved as auto- or alloantibodies, according to the ABO group of the antibody producer.

In 3 cases, 2 group O and 1 group A2 blood donors, both polyclonal and monoclonal ABO typing reagents, gave false-positive reactions with unwashed red blood cells (RBCs). AB sera and autocontrol gave negative reactions. The true ABO group was easily determined after washing the RBCs at 22°C. These results indicated that the donor’s serum and an additive in commercial reagents were involved in the agglutination reaction. In the 4th case, a group A1B patient, the plasma from an EDTA-anticoagulated blood sample, agglutinated A2 and O RBCs, whereas the serum was inactive.

In the 4 cases, the serum was shown to react with random group O RBCs only in the presence of appropriate concentrations of citrate or EDTA solutions. The results of a typical experiment are shown in table 1. In agreement with the work of Beck et al. [4], the serum was reactive in citrate or EDTA concentrations exceeding those required for full chelation of Ca2+. For example, although when added to the serum of our A2 blood donor, 2.5 mM oxalate alone was unable to produce an agglutination, it chelated Ca2+ and, as a result, allowed

<table>
<thead>
<tr>
<th>Additive, mM</th>
<th>Citrate</th>
<th>EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>512</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>512</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>128</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>128</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>256</td>
</tr>
<tr>
<td>1.5</td>
<td>0</td>
<td>512</td>
</tr>
<tr>
<td>0.7</td>
<td>0</td>
<td>128</td>
</tr>
<tr>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Full chelation of Ca2+ between 0.7 and 0.3 mM.

Table 1. Agglutination titers of O RBCs by the serum of a group O donor at various concentrations of calcium chelators
lower EDTA concentrations to be active. Thus, these antibodies were dependent on the presence of free calcium chelator molecules rather than on the absence of ionized calcium. EDTA was much more efficient as a cofactor than citrate since its optimal concentration was 10–20 times lower. Divalent cations (CaCl$_2$ and to a lesser extent MgCl$_2$) were able to prevent the effect of chelators or to disperse preformed agglutinates. Thus, carboxyl groups were the functional part of EDTA and citrate molecules.

These properties were used to prepare eluates in the two group O and the A$_3$ individuals. One volume of washed O RBCs was incubated for 1 h at 22°C with 5 vol of serum and EDTA in saline at a final concentration of 3 mM. The sensitized RBCs were then washed 4 times with 1.5 mM EDTA in saline. Elution was achieved by adding saline containing CaCl$_2$ at a final concentration of 10 mM. A reactive eluate could not be obtained when RBCs were washed in EDTA-free saline. This finding clearly also showed that EDTA was a direct cofactor of the binding of the antibody to RBCs.

The antibodies were tested against O, A$_2$, A$_1$, Oh, Oi adult RBCs and against Oi cord RBCs. They showed either anti-H (2 cases) or anti-HI (2 cases) specificity. In the case reported by Beck et al. [4], the antibody specificity could not be determined.

References

Dr. D. Janvier
Site Transfusionnel Saint-Louis
1, av. Claude-Vellefaux
F–75475 Paris Cedex 10 (France)
Authors’ Reply

P.L. Chiodini, P.E. Hewitt, J.A.J. Barbara
National Blood Service, North London Centre,
London, UK

We are grateful for Dr. Gillon’s thoughtful contribution to the discussion of malarial antibody testing of blood donations. The following comments address his concerns.

The most important objective is to exclude donors with asymptomatic parasitaemia, especially those due to Plasmodium falciparum, as the principal risk for blood recipients is from partly immune, symptomless carriers of malaria [1]. Donor selection procedures relying on history taking and exclusion periods can break down. Failure in history taking led to 2 cases of transfusion-transmitted falciparum malaria in 1988 [2] and 1994, as described in our paper. Malarial antibodies were readily detectable in both donor sera after the event; antibody screening would have prevented both cases.

Until recently, malarial-antibody-positive individuals were donating blood after the exclusion period, simply because their sera were not tested. In a 1997 fatality from transfusion-transmitted falciparum malaria [unpubl. data], the donor had reported returning from a tropical area 3 years previously and thus fulfilled the relevant donor criteria at that time. Upon investigation, as asymptomatic donor was found to have malarial antibodies. Donor antibody testing would have prevented this transmission.

Introduction of antibody testing provides an additional safeguard, whether or not the current tropical area exclusion period is shortened to 6 months. Symptomatic parasitaemic patients will be excluded from donation. Voller and Draper [3] draw a clear distinction between a non-immune person with an initial malarial infection which is rapidly and radically treated and who may develop only transient low malarial antibody levels, and a resident of a malaria-endemic country with repeated infection, who receives only minimal treatment. The latter is likely to develop high antibody levels with a very wide spectrum of reactivity which may persist for years after the individual leaves the malaria-endemic area. In endemic areas, where patients are repeatedly re-infected, low or inapparent parasitaemias still elicit strong serological responses [3]. Thus, asymptomatic parasitaemic individuals are expected to have high titres of malarial antibodies [4]. ELISA, although slightly less sensitive than IFAT in our study, still represents a major improvement over no routinely available malarial antibody testing, which is the situation prevailing until now. The 2 patients who were anti-malarial IFAT positive, ELISA negative, in our study would have been deferred from donation by a 6-month exclusion period, even though the ELISA failed to detect malarial antibodies in the month after leaving a malarious area. Although it is useful to know the sensitivity of the ELISA in acute malaria, it is not intended for that purpose and we have not advocated its use in that context.

Presentation beyond a 6-month exclusion is expected to be more common for Plasmodium vivax than P. falciparum, because P. vivax has a hypnozoite stage in its life cycles, which can initiate a delayed primary attack of malaria [5] while P. falciparum has not. Even a 1-year period would fail to exclude all cases of P. vivax, as some strains can present with a primary attack after 18 months [3]. However, a first infection with P. vivax is highly likely to be symptomatic. A person with chronic asymptomatic vivax infection will have formed antibodies, which cross-reacts to some extent in the commercial ELISA – an improvement on the current situation without antibody testing.

The figure of 40,000 units is indeed a crude estimate, but there is no doubt that a significant amount of blood is being wasted. In 1985, Wells and Ala [1] stated that the tropical area donor deferral policy resulted in the exclusion of 5.8% of West Midlands volunteers, equivalent to 12,000 potential donations per year. Since 1985 more UK travellers are visiting malarious areas.

We advocate screening of tropical area donors by history taking, antibody testing, and a 6-month exclusion period. Relying solely on history taking can cause confusion and concern, with highly motivated donors being turned away unnecessarily, and errors in the donor exclusion process. The antibody test provides and added level of protection and simplifies a complex situation. It is intended to complement, not replace, history taking. If such a system is used, there is a potential for a significant increment in the number of individuals eligible to donate blood. Furthermore, a clear and encouraging message is being sent to donors – they will not be turned away without good reason.

References


P.L. Chiodini
North London Centre
Colindale Avenue
London NW9 5BG (UK)
Tel. +44 181 258 2700
Fax +44 181 258 2970