Evaluation of Complement Activation on Cardiopulmonary Bypass and in Retransfused Oxygenator and Shed Mediastinal Blood

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Abstract

Several methods of blood salvage are used in cardiac surgery. Our aim was to study, on the basis of complement activation, the quality of autologous blood products reinfused to the patient. Eleven patients with elective myocardial revascularisation were studied for: operative and cardiopulmonary bypass (CPB) data, hematology, blood chemistry and complement activation (C3a [des Arg] and terminal complement complex [TCC] or C5b-9 complex). Centrifugated oxygenator blood (COB) and shed mediastinal blood (SMB) samples were examined before reinfusion into the patient and the effect of this retransfusion on the systemic values.

The activation pattern for C3a [des Arg] and TCC was similar. Thus only evaluation of C3a [des Arg] is reported.

1. Anesthesia increased C3a [des Arg] two fold over baseline (85 +/- 64 vs. 161 +/- 103 ng/ml [standardised]; ns).

2. Accumulation of complement activation at 1 hr on CPB was 12 fold over baseline for C3a [des Arg]; 1984 +/- 677 ng/ml [standardised]; p< 0.01).

3. C3a [des Arg] in COB and in SMB were 5002 +/- 2027 and 4910 +/- 1985 ng/ml [standardised], respectively.

4. However, after retransfusion of each autologous blood product no further increase in anaphylatoxin concentration could be detected in the patient's blood.

We conclude that despite complement activation in reinfused autologous blood products no further increase is seen in the patient's circulation. This is explained either by the very potent clearing capacity of the body or the rapid absorption of C3a [des Arg] by cellular receptors.

Introduction

Cardiac surgery represents a major disturbance for the patient's homeostasis. This is mainly due to the long anesthesia, surgical stress, cardiopulmonary bypass (CPB) and administration of medications and blood products.

Recently a major effort has been made to reduce the use of homologous blood products due to the risk of transfusion transmitted diseases and reactions (1). In our clinic, in accordance with several reports, various procedures of post-operative blood salvage by retransfusion of autologous blood are routinely used, such as centrifugation of the oxygenator blood (COB) and retransfusion of the shed mediastinal blood (SMB) (2). We and others have focussed on the quality of such blood products (3). So far only a few reports have dealt with the alteration of the complement system (4).

Complement activation plays a major role in the immuno-regulation of the body and the pathway of activation consists of a classical and alternative route and the formation of the membrane attack complex (MAC, C5b-9) by the terminal complement components (5). We elected to measure accumulation of C3a [des Arg] and the terminal complement complex (TCC). Complement activation is provoked by contact of blood with artificial materials as they are used during CPB (6,7). The involvement of both activation pathways was described earlier during CPB (8,9). Bio-compatibility is most often judged by complement activation assessments.

The aim of this prospective study was to assess complement activation in 11 patients undergoing open-heart surgery as well as in the autologous and homologous blood products before retransfusion into the patient's circulation and the effect of such retransfusions for the patient's outcome.
**Method**

**Operative Procedure**

Eleven patients (9 males and 2 females) with a mean age of 56 years entered the study prospectively and randomly. All patients had one or more cardiovascular risk factors and seven out of eleven had a previous myocardial infarction. The operation consisted of elective myocardial revascularisation procedures with a mean of 3.18 +/- 0.26 distal anastomosis, always including one or more internal mammary artery grafts. Patients were operated under moderate hypothermia (26.7 +/- 1.3°C).

Intravenous (Fentanyl/Midazolam) and inhalation anaesthesia (Ethrane) was used. Anticoagulation before CPB was achieved with Heparin (4 mg/kg body weight) and was monitored by activated clotting times (ACT; Hemotech) and reversed by Protamin sulphate after CPB (Heparin/Protamin 1:1). Only membrane oxygenators (Maxima, Medtronic n=6 and CML, Cobe n=5) with cristalloid prime and moderate hypothermia (26.7 +/- 1.3°C) were used. The mean duration of CPB was 116 +/- 81 minutes with a cross clamp time of 67 +/- 26 minutes.

**Autologous Blood Products**

Post-operatively and routinely we retransfuse the centrifugated oxygenator blood (COB) and reinfuse within the first eight hours the shed mediastinal blood (SMB) from the chest drains. Details and quality assessment of these procedures have been reported earlier by our group and others (2,3).

**Laboratory Evaluation**

 Besides standard hematology (hemoglobin and hematocrit) and blood chemistry (sodium, potassium, calcium, protein and albumin) we examined complement activation (C3a [des Arg] and TCC) at the following intervals:

A. In the patient's circulation:

1. Pre-anesthesia
2. Post-anesthesia, before incision
3. After one hour of CPB
4. Arrival in the intensive care unit (2 hours 11 minutes after CPB completion)
5. After retransfusion of COB (4 hours 25 minutes after CPB completion)
6. After reinfusion of SMB (7 hours 12 minutes after CPB completion)
7. On post-operative day #1 (19 hours after CPB completion)

B. In the blood products

1. Centrifugated oxygenator blood (COB)
2. Shed mediastinal blood (SMB)
3. Homologous packed red cells (PC)

For measurement of complement activation the following assays were performed:

- C3a [des Arg], ELISA, (Progen, Heidelberg, Germany)
- TCC, ELISA using mouse monoclonal antibody aEll, which detects a neo-epitom on activated C9 (Mollnes, Norway) (10).

The results were standardised using the patient's serum concentration of IgG immunoglobulins from the first sample drawn as follows:

\[
\text{Complement concentration} \times \text{IgG of first sample drawn} \\
\text{IgG of the sample to standardise}
\]

For the autologous and homologous blood products standardisation was on the basis of the hemoglobin concentration.

Results are expressed as means +/- 1 standard deviation. Significance was calculated by the Student's T-Test.

**Results**

All patients were discharged from hospital in good condition. One patient, however, required a second emergency revascularisation due to acute graft occlusion with ventricular fibrillation and was therefore excluded from this report, thus leaving 10 patients in the study group.

The homologous and autologous blood requirements are summarised in Table 1. The mean total volume per patient of retransfused COB and SMB was 841 ml. This volume equals about 2.5 units of packed red cells, and therefore the further homologous blood requirement was relatively low with 1.8 units of packed red cells per patient. It is noteworthy that out of the ten patients, six did not need any homologous blood products. The mean hemoglobin concentration of all ten patients was 14.4 +/- 0.8 g/dl on admission, 9.9 +/- 1.1 on post-operative day #1 and 11.5 +/- 1.4 at discharge.

A. Complement activation in the patient's circulation

C3a [des Arg] and TCC showed a similar pattern of activation during the whole course of evaluation. Thus, hereafter, we shall only refer to the results of the C3a [des...
Arg] assessments.

Samples taken before and after anesthesia showed a doubling of the mean values (85 +/- 64 vs. 161 +/- 103 ng/ml [standardised]; ns). A significant (p<0.01) 12-fold increase was noted at 1 hr on CPB (1984 +/- 677 ng/ml [standardised]) as compared to the value after anesthesia. Thereafter the C3a [des Arg] levels decreased steadily over time to reach a value close to the upper limit of normal (200 ng/ml) 19 hours after completion of CPB (POD 1) (Fig. 1).

B. C3a breakdown products in homologous and autologous blood

The mean value for C3a [des Arg] in COB was 5102 +/- 2027 ng/ml [standardised] (n=8) and 4910 +/- 1985 ng/ml [standardised] (n=9) in the SMB as compared to 92 +/- 88 ng/ml [standardised] (n=10) in homologous packed cells (Fig. 2).

Discussion

Complement activation is considered by an increasing number of authors to represent a problem in open-heart surgery (11,12). Several adverse influences on the immunological system and the patient's homeostasis have been postulated (13,14) but are still not fully understood. The purpose of this study was not to determine which complement pathway is activated during cardiopulmonary bypass, but rather to evaluate the time-related complement activation of a patient undergoing cardiac surgery and to assess carefully complement activation in autologous blood. Not only the level of activation during CPB was of interest, but also the effect on the patient's circulation after retransfusion of COB and SMB. We found a significant increase in C3a [des Arg] values in both COB and SMB when compared to the values taken after one hour on CPB. This is most likely due to the additional blood handling and the long time during which the blood is in contact with artificial surfaces in vitro. The one hour CPB level of C3a [des Arg] is not to be considered as a peak value as other authors have shown its increase over time (15) during CPB.

The retransfusion of COB and the reinfusion of SMB did not raise the C3a [des Arg] levels when samples taken after completion of these procedures (Fig. 1) were analysed, a fact to attribute to the relatively small volume with high complement activation products as compared to the patient's own volume. In addition, the two autologous blood products were not given as a bolus but were reinfused over a time period of 1-2 hours. For this reason, our curves of declining C3a [des Arg] levels look similar to previously published reports where no autologous blood products were retransfused to the patient (16). This is either due to the body's ability to clear C3a rapidly or to the fact that it is immediately absorbed by cellular receptors.

In conclusion, the practice of retransfusing homologous blood products such as centrifugated oxygenator blood and shed mediastinal blood after cardiac surgery is apparently safe, helps to save blood and is validated by aspects of this study and results of our previous studies (2,3). Indeed, by applying these techniques, it was possible to perform cardiac surgery on six out of ten patients without any homologous blood products.

Acknowledgements

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References

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Questions and Comments

Q. In 1985 Dr. Ranginovish came out with a paper. He is a big advocate of washing shed mediastinal blood. He looked at complement products, specifically C3A, in shed mediastinal blood and in his series of patients he was able to show a removal of about 99 percent of the activated blood products. Your data here shows that, although you didn't wash your mediastinal blood, there was no adverse effect on the patient, as you summarized in your conclusions. He advocates washing mediastinal blood. Would you therefore not advocate washing mediastinal blood because of your data?

A. Well, I think that's the same problem as using a cell saver. If you would use a cell saver, of course you would get rid of most of the complement; as you would heparin, and most proteins, which then leaves you with washed red cells. I think it is probably the best method you can use if you want to have good autologous blood products. The problem is certainly the cost and time. You need a perfusionist who will be dedicated to do this job throughout the course of surgery. The other point we have to keep in mind is that all these procedures will reinfuse to the patient all his plasma proteins, which are of importance. If you use too much washed red cells you will be required to use fresh frozen plasma.

Gary Reeder, Denver, Colo.

Q. I have one question. Did I understand your data? Following anesthesia, you were collecting your samples for complement assays from the arterial line — was that correct?

A. That is correct.

Q. What do you think that contributed to your lack of demonstrating any increase following reinfusion of bloods due to the pulmonary absorption of the anaphylactoxic fragments that have reported to occur?

A. We would always use the same line for the whole procedure, except for the first blood which was by venipuncture. But I think due to the very high complement activation of our product, the point that you are mentioning might play a role, but not to the extent that it would bias the study.

Q. We have looked at some of this data with both C3A and C5AS, and find particularly in mediastinal shed blood levels of C3A as high as 14-15,000 nanograms per ml and a very significant reduction once it is reinfused and circulated through the pulmonary circuit. That's the reason for my question on that. Very interesting paper.

Sandra Pfefferkorn, Atlanta, Ga.

Q. I would like to ask you a small point regarding the complement activation you found between the Cobe CML and the Maxima hollow fiber. Were those numbers significant — I was not able to see the numbers, were they larger?

A. They were not significantly different. The numbers are small, I think 5 vs. 5, so we would not want to make any statement regarding this point.

Q. But surely from a numerical standpoint the numbers were higher for the CML.

A. Correct.

Gary Reeder, Denver, Colo.

Q. I have one question. Did I understand your data?

A. That is correct.

Q. What do you think that contributed to your lack of demonstrating any increase following reinfusion of bloods due to the pulmonary absorption of the anaphylactoxic fragments that have reported to occur?

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Fig. 1:
Complement activation in ten patients undergoing cardiac surgery. Complement activation was assessed by the formation of C3a [des Arg]. Results are expressed as mean values +/-1 SD in ng/ml [standardized]. The sampling times are:

1. Pre-anesthesia
2. Post-anesthesia, before incision
3. After one hour of CPB
4. Arrival in the intensive care unit (2 hours 11 minutes after CPB completion)
5. After retransfusion of COB (4 hours 25 minutes after CPB completion)
6. After reinfusion of SMB (7 hours 12 minutes after CPB completion)
7. On post-operative day #1 (19 hours after CPB completion)

Fig. 2:
Complement activation in homologous and autologous blood products before retransfusion into the patient’s circulation. Represented are the mean values +/- 1 SD of C3a [des Arg] for ten patients. The products are:

8. Centrifugated oxygenator blood (COB)
9. Shed mediastinal blood (SMB)
10. Homologous packed red cells
Homologous and autologous blood requirements during cardiac surgery and in the intensive care unit (mean +/- 1 SD; n=10)

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Table 1: Homologous and autologous blood requirements during cardiac surgery and in the intensive care unit (mean +/- 1 SD; n=10)