Author's response to reviews

Title: Alterations in plasma soluble vascular endothelial growth factor receptor-1 (sFlt-1) concentrations during coronary artery bypass graft surgery: relationships with post-operative complications

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Dear Editor

Thank you for your e-mail concerning our manuscript entitled "Alteration in plasma soluble vascular endothelial growth factor receptor-1 (sFlt-1) concentrations during coronary artery bypass graft surgery: relationships with post-operative complications" by Y. Denizot and coll. that we submit for publication in the Journal of Cardiothoracic Surgery. We are appreciative of the constructive comments made by all the referees. Their recommendations have allowed us to strengthen our manuscript. We send it back modified according to the comments. New sentences have been underlined in our revised manuscript.

Reviewer 0 (January 11, 2008)
The reviewer says that our study was done carefully concerning the storage and use of plasma samples. However he rejects our study firstly because we reported sFlt-1 levels as ng/mg total protein instead of pg/ml of plasma; and secondly because our study is not significant new as in a similar study presented three months ago in the same journal. Concerning the first point, a decrease of serum/plasma protein contents was observed during and after CABG surgery (Roth-Isigkeit and coll. Clin Exp Immunol 1999;118:242-246). To avoid the influence of haemodilution during bypass, sFlt-1 levels were expressed as ng/mg of total protein contents measured at the simultaneous times. These information have been added in our revised manuscript (page 6, lines 18-20, reference 16). Concerning the second point we firmly object. In our previous study (J Cardiothorac Surg 2007;2:38) we reported the release of sFlt-1 and sKDR during cardiac surgery and we focussed our attention on the putative mechanisms explaining these elevated sFlt-1 productions. We, thus, only investigated uneventful patients. In the present manuscript we have decided to compare
complicated and uneventful patients in the light of a recent report showing that sFlt-1 infusion improved cardiac and pulmonary functions in a mouse model of sepsis (Yano K and coll. J Exp Med 2006;203:1447-1458). We report that sFlt-1 release is markedly reduced in patients with cardiac or haematological impairments; a results that in our opinion is of importance regarding the role of VEGF on endothelial cell proliferation and neo-angiogenesis. For us, these results are new, of interest and deserve to be published.

Reviewer 1

Major points

1. In general less than 3 thaw-cycles had no effect on the interleukin and angiogenic growth factor plasma levels. All samples undergo exactly the same number of freeze-thaw cycles. Moreover all samples (from T0 to T5) were tested the same day. From our experience no significant differences were found for protein content (tested with the BCA Protein Assay Reagent) after 1 and 3 freeze-thaw cycles. Finally the sFlt-1 T0 values (in pg/ml) reported in our manuscript are similar like from those determined in plasma from healthy volunteers (Barleon et coll. Angiogenesis 2001;4:143-154). These comments have been added in our revised manuscript (page 4, lines 14-15; page 5, lines 20-21; page 6, lines 27-29, reference 17).

2. As requested ANOVA test has been used in our revised manuscripts (page 5, lines 24-26). Lowered plasma sFlt-1 levels were observed at the end of ECC in patients with haematological (p=0.001, ANOVA) or cardiovascular (p=0.006) impairments, but not with respiratory ones (p=0.053), as compared to patients with uneventful surgery. These comments have been added in our revised manuscript (page 2, lines 14-17; page 7, lines 4-9).

3. We apologized for the lack of clarity of our explanations. Sentences in our Material and Methods were misleading and have been deleted. Among the 31 patients, 15 had uneventful surgery and were weaned from the ventilator at the 24th post-operative hour. 16 patients had complicated surgery. 4 of them had transient vital complication but were weaned from the ventilator at the 24th post-operative hour. 12 of them had persistent vital complications and were not weaned from the ventilator at the 24th post-operative hour. These information are now more clearly indicated in our revised manuscript (page 6, lines 3-4, Table 1).

4. Yes. The following criteria were used for weaning patients from ventilator: PaO2/FiO2 > 300, hemodynamic stability without vasoactive drugs, complete rewarming of patients, no bleeding complications. These information have been added in our revised manuscript (page 4, lines 24-26).

5. Cardiovascular dysfunction was determined at T3, T4 and T5 according to the need of inotropic drugs to obtain a systolic arterial pressure above 90 mm Hg. Myocardial infarction and dysrhythmias were recorded. Pulmonary artery occlusion pressure, control veinus pressure and cardiac (and subsequent derived measures) output were monitored by a swan ganz catheter. At the time of the protocol systems monitoring blood volume by analysis of arterial curve (such as Picco or Vigileo®) were not available. A reference for the Murray Lung Injury Score has been provided. These information have been added in our revised
6. No significant difference could be documented between the two groups on several parameters including age, gender ratio, weight, body surface area, ejection fraction, preoperative left ventricular end diastolic pressure, number of grafts, ECC duration, heparin total dose, protamine total dose, preoperative blood infusion and blood reinfused by the cell saver. These information have been added in our revised manuscript (page 6, lines 13-17).

7. ECC duration and the number of grafts were not different between the two groups (page 6, lines 13-17). Concerning the severity of aortic atherosclerosis it is a possible hypothesis. However we have no experimental data or clinical values that can confirm this hypothesis.

8. Patients were selected on a consecutive manner according to the availability of investigators. Only patients with a preoperative ejection fraction >40% were included. This study was approved by the ethic committee of our hospital. All patients consented in an informed manner that samples might be use later in order to investigate the involvement of new factors. These comments have been added in our revised manuscript (page 4, lines 2-5).

9. We apologize for the lack of clarity of our discussion. Plasma VEGF levels significantly increased after CABG surgery and post-operative VEGF levels were higher in patients with cardiovascular and hematological impairments (Denizot and coll. Cytokine 2003,24:7-12). Results of the current study strengthen the hypothesis of a pathophysiological role for VEGF in mediating post-operative complications and that VEGF neutralisation in vivo appears beneficial to reduce the development of these post-operative adverse effects. However we admit that only a clinical study investigating the influence of sFlt-1 infusion on post-operative adverse effects after CABG might establish such a link. These comments have been added in our revised manuscripts (page 7, lines 24-29; page 8, lines 17-21).

Minor points
1. The correction has been made in our abstract (page 2, lines 7-9)
2. We indicated in our revised manuscript that no patients received corticoids (page 4, lines 23-24).

Discretionary revisions
1. It was the same protocol for all patients. Patients received 300 U/kg of heparin just before vascular cannulation. Incremental 100 U boluses of heparin were added each hour during the procedure to maintain an activated clotting time greater than 600 seconds. At the end of ECC protamine was administered in a 2:3 ratio with heparin. These information have been added in our revised manuscript (page 4, lines 18-22).
2. Yes. The blood was harvested from the surgical field with a cell saver and reinfused at the end of ECC to all patients (page 4, lines 22-23).
3. These information were not recorded in our study.
Reviewer 2

1. We agree with the reviewer. Our study did not directly establish a link of causality between sFlt-1 and adverse outcomes after CABG. However plasma VEGF levels significantly increased after CABG surgery and post-operative VEGF levels were higher in patients with cardiovascular and hematological impairments (Denizot and coll. Cytokine 2003,24:7-12). Results of the current study strengthen the hypothesis of a pathophysiological role for VEGF in mediating post-operative complications and that VEGF neutralisation in vivo appears beneficial to reduce the development of these post-operative adverse effects. Only a clinical study investigating the influence of sFlt-1 infusion on post-operative adverse effects after CABG might establish such a link. These comments have been added in our revised manuscript (page 8, lines 17-21). Two mechanisms can explain the role of sFlt-1. For the first one, sFlt-1 can directly neutralize circulating VEGF. In the second one, sFlt-1 can inactivate VEGF through sFlt-1 binding to membrane-bound Flt-1 and KDR receptors. We favour the second hypothesis since VEGF levels picked at the 6th and 24th post-operative while sFlt-1 levels picked during ECC. These kinetics of VEGF and sFlt-1 release might indicated a similar sFlt-1 mechanism for the diverse outcomes observed (cardiovascular and haematological impairments). These comments have been added in our revised manuscript (page 8, lines 7-14).

2. As indicated in our ¿Study population¿ section (page 3, lines 28 and 29; page 4, lines 4 and 5) all samples were collected 12 years ago for a clinical study investigated the role of lipid mediators, cytokines and growth factor during and after CABG. This study was approved by the ethic committee of our hospital. All patients consented in an informed manner that samples might be use later in order to investigate the involvement of new factors.

3. We agree that aprotinin may have modified the inflammatory response of these patients. However all patients (both uneventful and complicated) received aprotinin. Thus, it is not the use of aprotinin that reduced sFlt-1 release in complicated patients. These comments have been added in our revised manuscript (page 7, lines 11-15, reference 18). In this study we investigated CABG surgery with ECC. In the last few years, off-pump coronary artery bypass grafting has gained widespread attention as an alternative technique to conventional on-pump coronary artery bypass grafting. However the available literature does not permit definitive conclusions to be made on the advantages of off-pump surgery with respect to the systemic inflammatory response. Moreover mortality, stroke, myocardial infarction and renal failure were not improved. Clearly investigation of sFlt-1 and VEGF release during off-pump cardiac surgery would be of interest to ensure the involvement of angiogenic/anti-angionic pathways during this type of cardiac surgery. These comments have been added in our revised manuscript (page 7, lines 15-22, references 19, 20).

4. There was no sample size calculation. This is an observational study for which no previously published data was available to perform it. As requested results are now presented as mean ± SD

5. Commercial sFlt-1 and sKDR ELISA kits were only recently available. Until
now only one paper has investigated sFlt-1 during cardiac surgery. In this study we reported, for the first time, the release of sFlt-1, but not sKDR, during CABG surgery with ECC. In this previous manuscript we focussed our attention on the putative mechanisms explaining these elevated sFlt-1 production. We, thus, only focussed on uneventful patients. In a second set of experiments we have decided to test complicated patients in the light of the recent report showing that sFlt-1 infusion improved cardiac and pulmonary functions in a mouse model of sepsis (Yano K and coll. J Exp Med 2006, 203:1447-1458).

Reviewer 3
As suggested we presented clinical postoperative data as a table (page 6, line 2, Table 1).

Hoping our revised manuscript will find its way in your journal,

Yours sincerely

Dr. Yves Denizot