Author's response to reviews

Title: Juvenile elastic arteries after 28 years of renal replacement therapy in a patient with complete Complement C4 deficiency

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Author's response to reviews: see over
First of all, we would like to thank the reviewers for their efforts and valuable comments on our manuscript. These helped very much to improve the revised version. Please see the detailed point-by-point response below with the points raised by the reviewer written in black letters and our response in red. Corresponding major changes in the manuscript are printed in red letters.

Reviewer: Ari Mennander

Reviewer’s report:
I thank the Editor for the privilege to review this interesting case report. I hereby suggest for minor essential revision prior to publication:

1. Please consider to reduce your number of figures included. I would delete figures 2, 3, 5 and 6, since the data is provided on the text.

We agree that the number of figures is quite high. According to the advice we deleted figure 2b and figure 4a. To illustrate not only the lack of calcification but also the persistence of elasticity we would like to keep figure 2a – now named figure 2 - and figure 4b – now named figure 4 - in the manuscript.

2. On Discussion, last but 2nd sentence, 1rst paragraph: “That these mechanisms...” Please explain or rephrase for clarity.

We rephrased as follows on page 6, line 9-10: “The efficacy of these mechanisms is clearly illustrated by the calcifications of coronary and peripheral vessels”.

3. Lack of complement C4 was detected in serum in this patient. Are we to be certain that there is also a lack of complement in tissue as well? Would the authors have tissue biopsy available? Please comment.

Staining for C4 was performed in the biopsies of the patient’s own kidneys and of the transplant. Deposits of C4 were not detected demonstrating that consumption of C4 did not occur.

We added on page 3, third from last to last line: “Immunofluorescence of the biopsies of the patient’s own kidneys and the transplant showed granular deposits of IgG, IgM, IgA and C3 in the mesangium and along the glomerular capillary walls. Staining for C4 was negative.”

4. Though the lack of C4D may suggest protection against atherosclerosis, it may also signify additional risk for other pathologies, such as dissection. I would add text in Discussion to speculate on this possibility (Lack of C4d deposition may reveal susceptibility for ascending aortic dissection. Niinimaki E et al. Scand Cardiovasc J. 2012 Jun;46(3):177-82).

We thank the reviewer for the indication of this recently described and very interesting association and we added a citation and the following text on page 7, third from last to last line and page 8, first line: “Recently C4d deposits in the adventitia have been found in 50% of patients undergoing surgery of the ascending aorta. Lack of C4d staining was associated with aortic dissection. Whether complement may have a protective role against aortic dissection remains speculative.”

Reviewer: Juha Sinisalo

Reviewer’s report:
Florian Knoll et al. presents an interesting case report of 51 years old patient with two kidney transplantation, an unusual tissue type and total deficiency of C4. Patient has unexpected
course of atherosclerosis. This unusual presentation of a disease is worth publishing. However, minor essential revision is needed.

1) It seems that authors have published information about this patient previously (Ref 9), but not about athero-sclerosis, so this is a new approach. Authors have examined in that previous paper 6 more similar patients (who have complete C4 deficiency). Even though they are younger than now presented case, some of them have had kidney transplantation. Why not examine and report them all? It would make much stronger argument. I understand that this may be impossible, but should be considered. In deed it would be very interesting to examine all 6 patients presented in the previous paper. Only three of them developed ESRD and were transplanted. One of them died because of Kaposi sarkoma. Unfortunately we do not have current information on the remaining patient who is treated in Italy.

2) Examination of C4 deficiency from presented patient should be written in this paper more precisely. I understand that it is well done, but you should state that the C4 genes were tested, and the C4 was detected from serum (how many times and which intervals). Patient might produce some C4 and consume it --> there might be some detectable C4 in certain states of disease?

We re-described the mutation of the C4B genes on page 5, line 16-20: “This haplotype does not contain C4A genes, but instead two mutant short C4B genes with a 8127g→a point mutation at the donor site of the intron 28 splice junction, which abrogates correct RNA splicing[9]. Splicing according to a new potential donor site seven nucleotides downstream would lead to a stop codon in exon 29. These results and the absence of C4 from our patients serum strongly suggest that he was completely C4-deficient.” Testing for C4 from serum was done on multiple occasions. We added the information of multiple testing on page 5, line 11: “The patient’s primary disease is hereditary complete deficiency of complement C4, and C4 was never detected in his serum on multiple occasions.”

Consumption of C4 is unlikely as staining for C4 was negative in the biopsies of the patients own kidneys and the biopsy of the allograft. We added the information on page 3, third from last to last line: “Immunofluorescence of the biopsies of the patients own kidneys and the transplant showed granular deposits of IgG, IgM, IgA and C3 in the mesangium and along the glomerular capillary walls. Staining for C4 was negative.”

Concerning the association between the partial C4 deficiency, especially for C4BQ0 alleles and myocardial infarction, this seems to be not caused by partial C4 deficiency but by impaired function of neighbouring 21-hydroxylase genes. We want to point out that the specific allotype of our patient includes two functional 21-hydroxylase genes. (see also Ref. 10).

3) In contradiction to what authors present: it has been shown in some papers that C4 deficiency is connected to coronary artery disease (Int Immunol 2008:20;31-37, BMJ 1994:309:313-314, Molecular Immunology 1998;35:412, J Med Gen 202 39:46-51 Etc). Authors do not present anything about coronaries. Have they been evaluated? It could be so that coronaries act on complement differently than the “central arteries”? I would like to see a comment on that.

Till now the patient did not show any clinical signs of coronary artery disease. Recently a stress echocardiography did not show any signs of myocardial ischemia in the current pre-transplant evaluation. To elucidate this interesting question we asked the patient for permission to perform a coronary calcium scan. With an Agatston score of 782.5 the coronary arteries showed substantial calcification as expected for muscular arteries like the coronaries. Interestingly co-illustrated slices of the aorta were free of any signs of calcification. We added this
Computed tomography of the coronary arteries revealed severe calcification with an Agatston score of 782.5 corresponding to the 99th percentile of age and gender matched controls. Co-illustrated slices of the aorta were free of calcification.

4) Central arteries have been evaluated with CIMT and PWV. Thorax-x-ray must have been taken several times from the patient. Is there any sign of aortic calcification? If not, that would be an additional proof of lack of central atherosclerosis.

We re-evaluated Thorax x-rays of the last years and ultrasound investigations of the abdominal aorta and did not find signs of aortic calcification. Beyond that Echocardiograms did not show valvular calcification. This information was added in the manuscript on page 4, second to last to last line: “We did not detect signs of aortic calcification in thorax x-rays and in ultrasound investigations of the abdominal aorta and no signs of valvular calcifications in an echocardiography.”

5) When presenting a case of atherosclerosis, all cardiovascular risk factors should be shown: smoking? Cholesterol values are shown, but have they been stable? Patient has not used statin in any state?

The patient has been a non-smoker for his whole live up to now. Cholesterol values were in the same range for several years as the values shown in table 1 without using a statin.

6) Authors present just one case: conclusions should be cautious. Discussion, last paragraph, second sentence: Whereas atherosclerosis seems... I would use might.

We weakened our conclusion and used the term “might” instead of “seem” on page 8, line 5.

Reviewer: Joseph M Ahearn

Reviewer's report:
This is a truly remarkable case report by Knoll et al. The authors clearly describe a patient with Henoch Schoenlein Purpura who had been on renal replacement therapy for 28 years. The extraordinary observation is that this patient, despite a full range of risk factors for vascular damage, demonstrated carotid artery intima media thickness below the normal range and normal pulse wave velocity. In contrast the patient's peripheral muscular arteries were heavily calcified. The authors attribute this unusual finding to an inherited complete lack of complement C4 protein due to genetic mutations. Normal elastic arteries despite prolonged renal replacement therapy in this patient is suggested to support the hypothesis recently proposed by Shields et al. that direct binding of C3- and C4- activation fragments to elastin and collagen fibers in the vascular wall of otherwise healthy individuals may contribute to the development of vascular stiffness. The report could be improved by addressing the following points:

Discretionary Revisions

1) In the introduction, it is stated “no effective therapeutic strategy has been identified today until today”. This statement suggests that an effective therapy has now been identified. This is misleading and should be rephrased.

We rephrased as follows on page 3, line 4: “No effective therapeutic strategy is available to prevent or slow down vascular calcification and arterial stiffening in patients with chronic kidney disease.”

2) It would be interesting to learn more about the pathology reports from the patient's renal tissues that may have been biopsied and/or removed. In particular it would be interesting to
know the patterns of complement deposition that were observed both in the patient’s autologous tissue and in the failed allografts.

Complement C3 and IgG, IgM, IgA formed granular deposits in the mesangium and along the glomerular capillary walls in both the patient’s own kidneys and the allograft. Staining for C4 was negative. We added this undeniably important information on page 3, third from last line: "Immunofluorescence of the biopsies of the patient’s own kidneys and the transplant showed granular deposits of IgG, IgM, IgA and C3 in the mesangium and along the glomerular capillary walls. Staining for C4 was negative."

Reviewer: Walter Speidl

Reviewer’s report:

This is an interesting case report describing a patient with a very rare disease, namely complete C4 deficiency. However, the statement that the patient has “juvenile” elastic arteries based only on duplex ultrasound of the carotid arteries and measurement of carotid-femoral pulse-wave velocity is an overstatement.

In addition to the duplex ultrasound of the carotid arteries and to the cf PWV measurement no signs of calcification were detectable in thorax x-rays, in ultrasound investigations of the abdominal aorta and in co-illustrated slices of the aorta when performing the coronary calcium score (see below). This information was added in the manuscript on page 4, second to last to last line: "We did not detect signs of aortic calcification in thorax x-rays and in ultrasound investigations of the abdominal aorta and no signs of valvular calcifications in echocardiography."

Therefore we take the view that is justified to maintain the term "juvenile".

Major Compulsory Revisions

As the patient was evaluated for kidney transplant, one would expect the he has undergone coronary angiography. Authors should include data about calcification of the coronary arteries. If coronary angiography is not available, at least a coronary calcium scan should be performed.

The patient did not show signs of stress induced coronary ischemia in a stress echocardiography. Hence no coronary angiography was performed in the pre-transplant evaluation.

We thank the reviewer for the suggestion to perform a coronary calcium scan as it provides important and easily available information. Therefore we asked the patient for permission to perform a coronary calcium scan to quantify the intensity of coronary calcification and as stated above an Agatston score of 782.5 showed substantial calcification. As the coronaries are muscular arteries this result supports the theory that elastic but not muscular arteries were protected from calcification due to complete deficiency of complement component C4. We added this information on page 5, line 6-8: "Computed tomography of the coronary arteries revealed severe calcification with an Agatston score of 782.5 corresponding to the 99th percentile of age and gender matched controls. Co-illustrated slices of the aorta were free of calcification."