Author’s response to reviews

Title: Low serum IGF1 is associated with hypertension and predicts early cardiovascular events in women with rheumatoid arthritis

Authors:

Malin Erlandsson (malin.erlandsson@rheuma.gu.se)
Lovisa Lyngfelt (lovisa.lyngfelt@gu.se)
N Åberg (david.aberg@medic.gu.se)
Caroline Wasén (caroline.wasen@rheuma.gu.se)
Rachelle Espino (w4m29@students.keele.ac.uk)
Sofia Silfverswärd (sofia.silfversward@rheuma.gu.se)
Mitra Nadali (mitra.nadali@vgregion.se)
Katharina Jood (katarina.jood@neuro.gu.se)
Karin Andersson (karin.andersson@rheuma.gu.se)
Rille Pullerits (rille.pullerits@rheuma.gu.se)
Maria Bokarewa (maria.bokarewa@rheuma.gu.se)

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The comments including 2 figures and a table that lost its formatting are available as a word document uploaded as personal communication

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Low serum IGF1 is associated with hypertension and predicts early cardiovascular events in women with rheumatoid arthritis

Malin C Erlandsson, PhD; Lovisa Lyngfelt; N. David Åberg, MD, PhD; Caroline Wasén; Rachelle Espino; Sofia Töyrä Silfverswärd, PhD; Mitra Nadali, MD; Katharina Jood, MD, PhD; Karin ME Andersson, PhD; Rille Pullerits, Md, PhD; Maria I. Bokarewa, MD, Professor
Dear Alessandro Recchioni,

Thank you for giving us a possibility to revise and improve the manuscript indicated above.

Following recommendations of the two Reviewers, we have now addressed the comments and modified the text of the manuscript as appropriate. Find below our point-by-point responses to the Reviewers and the revised version of the manuscript.

We have added a table with clinical characteristics of RA patients that also includes information on treatment in the new Figure 2A.

We have attached a graph illustrating close correlation between the CVR estimated by the Framingham algorithm and SCORE to justify our choice of the Framingham algorithm in this study.

We have explained the discrepancy in statistical results presented in Table 1 and Figure 1E. We have also modified the text and a header in the figure to improve the clarity of presentation.

We have expanded the Introduction with more references on associations between hypertension and IGF1.

We have added the conclusion section at the end of the discussion.

We believe, that this improved version of the manuscript corresponds to the requirements of the journal and is sufficient to be accepted for the publication.

Gothenburg, June 12th, 2019

Sincerely,
Reviewer reports:

Reviewer #1 Michael Nurmohamed: This is an interesting manuscript that indicates that low IGF1 levels are associated with CVD in RA and linked to hypertension and aberrant IGF1 receptor signalling.

Comments

Abstract: please explain the abbreviation IGF1

This is now transcribed

"Longitudinal cross-sectional" seems in contradiction

Both types, longitudinal and cross-sectional are observational studies. Our study has two parts, it starts as a cross-sectional and the estimation of CVR is done on the measures collated at baseline. New cardiovascular events have been registered continuously during the period of 5 years and allows to longitudinal follow the consequences and accuracy of the CVR estimation.
We have now modified the title of the manuscript, which now reads “The longitudinal observational study”.

P 3, Line 12: stroke risk: absolute or relative (?), I assume the latter

Due to the prospective nature of this study we could calculate both, the absolute risk (defined by the number of events in each group divided by the number of people in the group) and the relative risk (defined as a proportion between the absolute risk in IGF1low group divided by the absolute risk in the IGF1 high group). In this study IGF1hi group had the absolute risk of 2.3% and IGF1low group had the absolute risk of 10.4% giving the relative risk of CV event 3.47 times higher for the patients in the IGF1low group. This information is now added to the text of the manuscript in page 8.

P 3, Line 27, please add reference for "blood volume"


P 3, Lines 43-52, please explain why the older studies indicated that higher IGF1 levels were associated with increased CVD risk, whereas the newer studies indicated the opposite

This is a controversial issue. Our understanding is that the difference is explained by different study cohorts. Recent studies focus on general population with low prevalence of obvious endocrinological conditions “Schutte AE, Hypertension 2014”, while early reports studied patients with congenital or acquired abnormalities in GH/IGF1 production. “Bondanelli M. Pituitary 2001” and Vitale G, Clin Endocrinol (Oxf). 2005. This explanation is now added to the introduction section page 4 and to the reference list.

P 4, first paragraph: why was ischemic heart disease not considered?

Indeed, premature ischemic heart disease has attracted attention in female RA patients. During the 5y follow up only 1 patient developed MI, thus the study is underpowered to address this question. Also, we have no information about subclinical ischemic heart disease in our study cohort.

P 4, M&M: Please add the sample size considerations, were the 184 patients consecutively enrolled?
Yes, this is a consecutively enrolled RA cohort. This is now added in the methods section on page 5.

P 4, lines 46-51, Why was "SCORE" not used, It's validated for most European countries. How do the results change when SCORE is used?

The reason we use Framingham algorithm is that it has been the first to develop and serves today as the reference CVR calculator in the scientific community worldwide. It is widely used and has been repeatedly validated for CV risk prediction not only for ischemic heart disease and stroke, but also for atrial fibrillation, congestive heart failure, diabetes mellitus, fatty liver disease, etc. The CV risk calculations are performed with 5 different algorithms including Framingham 10 year risk (BMI and lipids, presented in the manuscript), and also SCORE, modified (m)SCORE, QRISK®2-2014 10 year risk, ACC/AHA ASCVD 10 year risk. The obtained results showed a remarkable consistency with high level of correlation (r vary between 0.779 – 0.921 for IGF1low group and 0.806 – 0.936 for the IGF1hi group). Find below the figure to illustrate correlations between SCORE and Framingham results in the total study cohort. You may appreciate in this figure that Framingham calculation gives several subdivision tracts for each value of the SCORE calculator, which permits identifying smaller distinctions in CVR.

## Two figures are missing, see the uploaded word document##

P 5 Lines 2-3. The BP burden: this is rather new, please add reference. Moreover, I wonder if mean arterial pressure would not be better.

We have done calculations using BP burden (simple addition of SBP and DBP values) and mean arterial pressure ((SBP+DBP)/2). The results are identical and indicate the same statistical differences.

P 5 Serological measurements: two different methods for IGF1 determination were used.

The reason why we have two IGF1 methods is obvious – we have two cohort studies. However, IGF1 is a stable peptide and the analysis is considered to have quite little methodological problems with little inter-assay variation, which we may stress. I give one reference on that matter “Burns C, Growth Horm IGF Res. 2009”.

P5 Line 54: please explain the choice for 2 groups instead of 3 or 4 groups, as e.g. the contrast might be higher when the highest quartile is compared to the lowest quartile.
The reviewer is most probably right in his suggestion; the comparison of the lowest and highest quartile of IGF1 could show larger differences in CVR. The decision to use median value to split the cohort is based on practical reason of clinical significance of “normal” low rather than extreme low IGF1 levels. Additionally, the analysis of quartiles requires larger cohorts. Potentially joint efforts of several European cohorts may be combined for such an analysis. The size of our cohort will be strongly underpowered by analysis in quartiles.

P 6 Line 6 what's "shorter stature"

It is a typo and should stay “stature”. Short stature refers to a height of a human being short. In this study, short stature is defined as a height <165cm. To avoid misinterpretation, the “shorter stature” is now substituted with “lower in height”.

P 6 line 43, please note that LDL and HDL were not statistically different according to Table 1

Indeed, table 1 presents comparison between the RA and IS cohorts, in addition to the groups with low and hi IGF1. Figure 1E shows the RA patients aged <50y, and in this subgroup the blood lipids are significantly altered. We agree, this subdivision was not so clearly described. We have now added a title to the figure 1E and added in the results text on page 7.

P 7, second paragraph: actually part of this belongs to the methods section

I am uncertain, what the reviewer means. We now added clinical information of RA patients in a new figure (Figure 2A in revision).

## The table lost its formatting, see the uploaded word document##

n=184 Median [IQR]

Duration of RA, years 7 [4-14]

ACPA/RF positive, n (%) 164 (89%)

DAS28

Tender joints, n
<table>
<thead>
<tr>
<th>Swollen joints, n</th>
<th>ESR, mm/h</th>
<th>3.03 [2.09-4.06]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 [1-4]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 [1-3]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 [6-13]</td>
<td></td>
</tr>
</tbody>
</table>

Remission

<table>
<thead>
<tr>
<th>DAS28≤2.6, n(%)</th>
<th>No swollen joints, n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>68 (37%)</td>
</tr>
<tr>
<td></td>
<td>60 (33%)</td>
</tr>
</tbody>
</table>

MTXmono, n

MTX+DMARD, n

MTX+TNFa inhibitors, n

MTX+Other biologics, n

No DMARDs, n 82

47
30
15
10

Oral glucocorticoids, n

Dose, mg/day 21 (11%)

5 [5-5]

P 9, line 43-47: does this still hold when comparing RA patients in remission?
At baseline, a substantial part of the RA patients was in remission (see Figure 2A of this revision). Excluding patients with active disease (DAS28>3.2) does not change the outcome, but compromises the statistical power due to the fact that the patients with active disease are also older (median age 55.0 vs 50.5, p=0.043), while major differences are observed in the patient groups <50 years.

P 10 Another limitation is the male RA patients were not studied. Furthermore, that two different methods for IGF1 assessment were used.

Certainly, our study comprises no male RA patients. This is now outlined in the limitations of this study in page 11.

Regarding the two different methods, see our response above.

Reviewer #2 Patrick Dessein: Reviewer comments

Comments:

In this study, Dr Erlandsson and colleagues document that low IGF 1 levels predict particularly hypertension related cardiovascular events in RA. Cluster analysis revealed a link with altered IGF 1 receptor signalling. This investigation is elegantly designed. The data analysis is appropriate and the results are presented clearly. This study has potentially important implications in our understanding of cardiovascular risk as well as other complications (e.g. rheumatoid cachexia) associated with RA.

I have a few comments that may help to further enhance the manuscript.

How many patients were on glucocorticoids and did this intervention have an impact in the present context?

This information is now added in Figure 2A.

Results, page 6, third paragraph. It is stated that hypertension was the most prominent distinctive feature as relates to associations with low IGF 1 levels.
However, Figure 1C also shows that associations with obesity and high cholesterol concentrations appeared as strong. Also, the association with HDL cholesterol was not significant, this in contrast to what is stated.

Table 1 shows that levels of TC, HDL and LDL are significantly different between RA and IS groups with low IGF1. Thus, these parameters are not in common for the two cohorts. These differences between RA and IS cohorts are most probably explained by use of statins by all IS patients.

The levels of TC and HDL show inconsistent results when IGF1lo and high groups are compared due to the fact that different statistics was applied. Figure 1E shows the results obtained in RA patients <50y. This explains the difference between Table 1 and Figure 1E. This was not so clearly described in the text. We have now added a title to the figure 1E and added in the results text on page 7.

Similarly, in Figure 2B, combination DMARD and TNF alpha inhibitor treatment did not appear significantly associated in contrast to what is mentioned in the text on page 8.

Sorry for this. Patients with lowIGF1 are significantly often treated with MTX monotherapy, while the number of patients treated with combination of MTX+TNFa or biologics was similar in both groups. This is now corrected on page 8.

Discussion, on page 10. It is stated that glucose intolerance and type 2 diabetes are rare in studied RA patients. Type 2 diabetes was indeed uncharacteristically low in the present RA cohort. This may deserve further comments. Maybe it is because the BMI was also somewhat low and perhaps very few patients were on glucocorticoids?

Indeed, the median BMI is 25.9 and 24.0 in the IGF1low and 23.9 and 24.6 in IGF1hi groups of RA and IS cohorts, respectively. These BMI numbers are representative and similar to general female population of Sweden (BMI 24.3; European Commission, Eurobarometer 59.0). Several other explanations are usually proposed for a high frequency of glucose intolerance, e.g. inflammation is one of them, use of glucocorticoids is yet another one. These traditional explanations do not really fit our RA cohort. We are puzzled by high frequency of glucose intolerance in RA patients and are intended to study this issue with all attention in future.

It may also help to add a concluding paragraph summarizing the main study findings and their implications for studies and future patient management.
Thank you, we have now summarized the main findings of this study in perspective of future patient management.