Author's response to reviews

Title: A Reference-frame for blood volume in children and adolescents.

Authors:

   Ann M Raes (ann.raes@ugent.be)
   Sara Van Aken (sara.vanaken@ugent.be)
   Margarita Craen (Margarita.Craen@ugent.be)
   Raymond Donckerwolcke (Rdo@skin.azm.nl)
   Johan Vande Walle (johan.vandewalle@ugent.be)

Version: 3 Date: 7 November 2005

Author’s response to reviews: see over
Dear Mr Chairman,
Dear editorial board.

We are pleased that the editor and the reviewer considered the manuscript of interest to readers of BMJ. Although we understand and expected many of the comments made by the reviewer, we think that we have enough arguments to defend this paper, and have done our best to include all answers into the paper. Of course we had to concentrate our answers in the text, because of the limited place. We had to reduce the “background”, by reducing the parts that are retaken in the discussion, to avoid a too long article and doubling up information.

In the following pages we are given a point to point defense on the comments of the reviewer, referring to what we have adjusted in the text.

General comment
As we mentioned in our paper we expected the comments made by the reviewer. We do agree with his comments. The major criticism of this study is the fact that we studied a population with steroid sensitive nephrotic syndrome during long standing remission. The aim of the study is an extension of a previous study performed in our centre. The most important reason to extend this previous study is listed here.

- The limited number of data to establish a reliable reference frame. This problem is solved in the present study.
- The small number of adolescents during puberty in different pubertal states. This makes it difficult to objectivate true sex-differences and to take conclusions.
- The “black gap” between the prepubertal children and the adult reference of P. Boer. And this was the main purpose of this study.
- Even if this reference frame will always be referred to as obtained in patients with steroid sensitive nephrotic syndrome in remission, the problem of the black gap is resolved.
We will address the different comments point-by-point

1) The studied population suffered from steroid responsive nephrotic syndrome. Can this population be considered normal, considering the effects of steroids on body composition and their underlying renal disease?

- Patients with cushingoid signs, growth retardation or drugs, possible interfering with blood volume regulation were excluded from this study. All children included, were 3 month’s steroid free. According to the recommended treatment of nephrotic syndrome the glucocorticoid dose is gradually tapered before stop. By this strategy most of the cushingoid stigmata are already regressed before stopping.

- Childhood steroid sensitive nephrotic syndrome (SSNS) remits completely in response to glucocorticoid therapy. Saha demonstrated that nephrotic children grew normally for their age before onset of the disease and growth remained normal despite prednisone treatment. The reason for this is that although protracted courses of glucocorticoids are often required, prolonged systemic inflammation is not a feature and in between relapses and steroid free times they grow normally.

- Only little information exists on body composition. Foster J et al. (in The American Journal of nutrition 2004) demonstrated that there is no relation between cumulative dose of glucocorticoids and body composition. It is known that body composition indicators increase or decrease relatively rapidly, so it is not surprising that body composition correlates poorly with lifetime glucocorticoid exposure.

- So we can conclude that the body composition in steroid sensitive nephrotic children,( in remission and steroid free since 3 months) does not differ from that of a healthy child.

- There is minimal confounding by underlying disease activity.

- Children with steroid responsive minimal change nephrotic syndrome are considered to have normal renal function (i.e. glomerular filtration rate (GFR), renal plasma flow (RPF) and renal water-and salt handling)

- Vande Walle et al. and Berg U. published data on renal function in patients with minimal change nephrotic syndrome in remission. Different methods to estimate
GFR clarified that GFR was within normal range and did not differ from renal function in normal children. Long-term follow up has shown no deterioration of the renal function.

- All our children had normal GFR, RPF, FENa, FEK, normal rennin activity and normal aldosterone levels) on the moment of the study. These values are added in table 1.

- All those patients have been followed for 4-6 years since the study on blood volume was performed and they still have a normal renal function and normal blood pressure. So, this finding can eliminate any suggestion of renal dysfunction that might have interfered with blood volume status at the moment of the study. (See table 1).

- So, there are valid data in literature to argue that a study population with SSNS in remission may be considered as a reliable and normal population. We believe that these arguments are robust enough to consider this subpopulation as a normal blood volume study population.

We have added in the document available extra data into the results (table 1) to demonstrate that there is no evidence that the patients may not be considered normal.
2) Why didn’t the authors use a genuine disease-free control population?

- We built upon a previous cohort of children with a steroid sensitive nephrotic syndrome in remission, although in a younger age category. To be able to include these data obtained in the previous study, one realizes that the same population with the same methods needs to be studied.

- Due to the “changing” ethical considerations over the past years, studies in a paediatric population, particularly in healthy children, are ethically not or difficult defendable if there is no direct or indirect benefit for the studied child. This is not the case “for” normal controls. However the understanding of the pathophysiology of the blood volume regulation has lead to important guidelines on the use of diuretics and albumin in the treatment of children with nephrotic syndrome. The second objection is the need for radiolabeled products to measure the different volumes. After finishing the study in the SSNS patients, we tried to get approval from the ethical committee to extend this study in a normal “population”, already anticipating the comments of every reviewer. However, the ethical committee refused this demand on the base of previous described reasons. Even if the ethical committee approved, methodological difficulties would raise, especially in the correct recruitment of normal children to participate in such an elaborated study, mainly for epidemiological reasons. How to collect a representative population sample, without bias?

- In addition, it is ethically not defendable to extend the present study to normal controls, because there are no evident reasons why the data cannot be considered as reliable and true normal values, except for some “theoretical” reasons. According to the EGCP guidelines and existing ethical guidelines, it is not ethical to perform studies, or redo studies in normal children, if enough data are already available and if these data are valid data.

  - These considerations and the expected comments of the reviewers are the main reasons why we waited so long to submit these data. The reason why we submit this study now, is because we are convinced, that it is not ethical to withdraw a valid reference frame, which is of importance for interpretation of hemodynamics and for comparing blood volume in various diseases such as hypertension, diabetes mellitus, chronic renal failure, transplantation and especially dialysis.
For many of these diseases alterations in blood volume can be learned from experiments in animal models, but they still remain animal models.

Studies in adults with the previous mentioned diseases have the methodological advantage

- that the patients are often considered to be homogeneous for body size and age
- and that studies in volunteer healthy controls are easy to obtain
- However it is not possible to extrapolate data from adults to children because of the large age-and size-differences and the changing body composition through puberty.

We have addressed this in the document in extenso

3) This is discussed, but are the arguments robust enough?

We believe that our arguments listed above are strong enough to overrule the comments. Although we cannot and will not neglect the methodological comments of the reviewer, we (as paediatric researchers) are convinced that not publishing these data, is withdrawing important information to refer to for different disease conditions in children in future research. In addition, there is no doubt that this information will likely not be available in a proper way (= in normal, unbiased selected paediatric population) in the next years.

We have addressed this in the document in extenso

4) The need to explain F-cell ratio more carefully.

The F-cell ratio is the relation between whole-body hematocrit and large-vessel hematocrit. This ratio is sensitive to changes in distribution of the circulation between the microvasculature (where the hematocrit is low due to the Fahreus –Lindqvist effect) and the large vessels, is not constant and has a large inter individual variability. This demonstrates that measuring blood volume by one single method (plasma volume and hematocrit using a fixed F-cell ratio of 0.91 derived from adult studies) is perhaps not
accurate enough and can lead to over–or underestimation of the BV. For this reason we calculated blood volume from measured plasma volume and red cell volume. . We have incorporated this answer into the text (the background, page 3)

- **We have addressed this in the document in extenso**

5) The authors question the ethics of using isotope measures in healthy children, but then report such a study in nephrotics in remission.

The critical difference lays in the ethical aspect. It is ethical difficult defendable to perform a study in a population of children, when there is no direct or indirect benefit from the study. However this study on blood volume in children with SSNS in remission and during nephrotic phase (data not incorporated in this paper) has lead to major direct and indirect benefit in the therapeutical approach of children during nephrotic state, namely by identifying the subgroups with hypo- or hypervolemia and thereby defining those who need a treatment with albumin or not, and diuretics or not. Because of the important clinical benefit, this study is ethically fully defendable.

The International Committee for standardization in haematology has recommended measuring red cell volume and plasma volume with radiolabeled methods. In absence of other validated methods, the combined measurement of red cell volume and plasma volume is the method of choice.

- **We have addressed this in the discussion in extenso**

6) The use of formulae to estimate LBM is criticised, but this study also uses this approach (albeit with a different set of formulae).

Presenting the data is always a difficult choice:

- LBM, a parameter to normalize vascular volumes, has been postulated as the gold standard since P. Boer has published it. . For this reason it is evident to use the same approach in this article, certainly if we want to compare the present results with previous articles, both in adults and children. Although we discuss the weak points of this normalisation parameter extensively, we were forced to conclude that LBM shows the highest correlation with blood volume, independent of sex-differences.
Adaptation of the formula is a practical consequence from the fact that children progressively pass from Turner stage I to V, and not acute.
Leaving “this part” out would have been the easiest solution... but it would not be correct

1. because it should make comparison with available publications impossible
2. because we would not emphasize the specific troubles during puberty. And particularly this subgroup, just the indication group where we wanted to extended our study population most.

We have addressed this in the discussion in extenso

7) Please explain the administration of potassium iodide
Potassium iodide is administered as prevention of thyroidal uptake of radioactive isotopes of iodine. Because this patients were administered 131 I-HAS we needed protection of the thyroid gland.

We have addressed this in the document in the methods

8): Please use either erythrocyte volume or red cell volume, not both.
We adapted the whole manuscript. We use red cell volume.

We have corrected this in the document

9) Results
Are the statistical differences in the ratio of volume parameters of body composition related to differences in red cell/plasma/blood volume or to differences in the derived body composition equations /normograms?
The highly significant differences by gender for V/BMI and V/BW for some Tanner stages and not other stages make one suspicious that the differences are not genuine, but represent statistical error. The differences by BSA make me suspect that the BSA normogram accounts for the differences. Could an expert medical statistician comment on this?

We have discussed this many times with an expert medical statistician (P.Boer), and have included the suggestion of the reviewer into the comment

This comment is correct, but the interpretation given to it, should be nuanced.
In adults all values are often plotted as individual values, thereby eliminating the methodological problem of normalizing. This facilitates statistical analysis enormously. However this does not mean that it not less biased, because even in adults there are body size differences.

The only publication on a large population normalizing for different parameters is the already many times mentioned publication of P. Boer. Our article is an extension of his findings in paediatric population.

The question whether the “statistical differences in the ratios of volume to various parameters of body composition are related to differences in red cell/plasma/blood volume or to differences in the derived body composition equations/nomograms “ is a theoretical “one”:

- Blood volume is “age, size, sex and puberty”-dependent. To be able to establish a reference frame, useful for studies in other disease conditions, you need to normalise.
  a. But multiple factors play a role. Many statisticians would prefer to perform a multivariate analysis, but this is not a correct way to perform statistics. Besides the gender-difference the other parameters (L, G, BSA, and LBM) are not independent.
  b. You could make percentile-charts, but we need a large group of children in every category for sex, length, age, pubertal state, something, which is not realistic.
  c. The other way to do is the way we have done it in a limited number of patients. We publish and statistics and the individual data. Although we try to conclude to the best normalisation-parameter, we give all objective information, to compare in future studies.

- However if in future studies in pathological conditions, patients have true hypervolemia, then they will be hypovolemic for all the demonstrated normalisation-procedures. if not, then we have to suspect that calculated statistical difference for the pathological patients, in one of the normalisation methods is due to body-size-differences

We have addressed this in the document in extenso
10) Is the correlation between blood volume and any parameter for size surprising? Would not you expect this relationship to be present (bigger individuals have a larger blood volume)?

It is obviously not surprising that blood volume is correlated with size parameters. It is general accepted that normative vascular fluid volume data should be corrected for body build and / or size. However insufficient data, concerning vascular fluid volumes, which employ accepted methodologies in children, are available. Additional value of this paper is the subdivision for different pubertal stages, because it is known that during the process of puberty hormonal influences can affect the body size parameters. Lean body mass tended to be the best body size index to predict blood volume, independently of age, gender or pubertal stage.

We have adapted this in the discussion document in extenso

11) The authors emphasise that one of the superior aspects of their study over previous data is that a fixed F-cell ratio has not been used, but has been calculated from the measured volumes. However they do not discuss differences between the measured ratio and the fixed ratio and how this influenced their results.

The fixed F-cell ratio, used by Linderkamp was derived from the mean value for F-cell-ratio in adult studies, that was 0.91 %, not taking in account the large SD.

Our data show clearly that the F-cell ratio in children is significantly lower 0,83 what is not surprising considering the different ratio between large bloodvessels / small bloodvessel-ratio. Using the 0.91 therefore would make statistical analysis and graphs more beautiful. but would not reflect the clinical reality.

We have addressed this in the document in extenso

12) The author offers valid reasons to use nephrotics in remission as their normal population. Can they clarify that prior steroid administration had not influenced body composition?

Body composition was evaluated in several studies of individuals exposed to exogenous glucocorticoids, including individuals with giant cell arteritis, systemic lupus erythematosus and renal transplant recipients. There was a significant increase in fat mass in these patients with long-term high dose exogenous administration of glucocorticoids. However children with minimal change nephropathy have different
regimens of glucocorticoids. Another difference is that children with SSNS have no systemic inflammation. Foster J et al. (in The American Journal of nutrition 2004) demonstrated that there is no relation between cumulative dose of glucocorticoids and body composition in these children. It is known that body composition indicators increase or decrease relatively rapidly, so it is not surprising that body composition correlates poorly with lifetime glucocorticoid exposure. So we can conclude that the body composition in steroid sensitive nephrotic children, in remission and steroid free for several months does not differ from that of a healthy child. So prior steroid administration has not influenced body composition.

We have addressed this in the discussion

13) The authors principle aim was to provide a normal range for blood volume in children. Do they need to provide regression equations that would allow others to derive a normalised blood volume for a child according to age and gender?

Strictu sense it is not obligatory to provide these regression equations. However, they are used to show that the best correlation is obtained between LBM and BV and that the intercept is the smallest one. In addition, there is no difference between males and females. Thus confirming the hypothesis that LBM is the best suitable body size parameter to normalize for independent of age, gender and pubertal state. Providing these regression equations can serve as an important tool for other investigators to compare their data with.

We have addressed this in the discussion in extenso

Conclusion:

We have tried to incorporate all the comments, or our defenses against the comments made by the reviewer, as much as possible in the text.

However we can not change the data, nor the study population, nor the limits of statistical analysis of these data. We are convinced that this data are the best available data on this moment, and that not publishing them would lead to an important loss of information, because it is very unlikely that in the next years another reference frame will be available. Studies in children are always very difficult, not only by technical
aspects (vascular access) and cooperation, but particularly by the need for normalization to compare data. This study delivers an important reference frame for pathological conditions. In absence of alternatives we try to convince you to publish it.

We have submitted this paper because to our opinion it was ethical not defendable not to submit important data for publication, if there is no alternative available.

We hope that our defense has convinced you

Many thanks

Dr Raes