ARTICLE DETAILS

TITLE (PROVISIONAL)	Mathematical modelling to restore circulating IGF-1 concentrations in children with Crohn's disease-induced growth failure: a pharmacokinetic study
AUTHORS	Sanderson, Ian; Rao, Arati; Standing, Joseph; Naik, Sandhia; Savage, Martin

VERSION 1 - REVIEW

REVIEWER	Dr Joanne Blair Consultant Endocrinologist and Honorary Senior Lecturer, Alder Hey Children's NHS Foundation Trust, Liverpool L12 2AP I have recruited patients to a study investigating the use of growth hormone in Crohns Disease. I am in discussion with the chief investigator of a study investigating IGF-I in the treatment of short children with Crohns Disease. We may also recruit patients to this study.
REVIEW RETURNED	14-Mar-2013

RESULTS & CONCLUSIONS	Please note that I am not qualified to comment on the mathematical methodology in this paper. It is important that the paper is also reviewed by an expertin this field.
GENERAL COMMENTS	I am not qualified to comment on the mathematical model described in this paper, and I will therefore limit my comments to the clinical aspects and utility of the model, recognising that some of these queries may have been addressed in the model. However, to aid the reader without the mathematical expertise of the authors, it would be helpful if they could be discussed more explicitly in the paper if this is so. Could the authors give more information regarding the reference data from which the IGF-I SDS are derived? IGF-I increases throughout childhood, with marked increases during puberty. In Crohns disease puberty is often delayed, and this is the case for some of the patients included in this study, for example LN08, who is 14.66 years old is prepubertal. If his baseline IGF-I SDS was derived from an age related reference data, it would be significantly lower than if it was compared to a reference set based on pubertal development. Likewise, if the target IGF-I SDS on IGF-I treatment was derived an age related, rather than a puberty related reference range, it may give an inappropriately high level for his stage of development. How have the authors dealt with the effect of puberty / pubertal delay on IGF-I levels? The low IGFBP-3 levels reported in the study subjects will have two

implications: (1) injected IGF-I will be cleared more rapidly from the circulation than in healthy subjects and (2) the ratio of free (i.e. biologically active) IGF-I: bound IGF-I will be higher in study subjects than in healthy subjects, from whom the reference data are drawn.

The investigators took alpha-1-antitrypsin to be a marker of protein losing enteropathy, which they speculate would be a marker of IGFBP-3 levels, and therefore investigated the relationship between stool alpha-1-antitrypsin and IGF-I clearance. They reported no relationship between these two measures. Was there any relationship between IGFBP-3 and IGF-I clearance? Is it correct that the mathematical model ensures that patients with an IGF-I SDS of <2.5 SD, based on measures of total IGF-I, also have a free IGF-I SD < 2.5 SD?

Finally, could the authors comment on their choice of an upper limit of IGF-I levels of +2.5SD? This upper limit is based on the observation that patients with acromegaly and IGF-I levels >2.5SD for ten years or more have an increased risk of bowel malignancy. Patients with Crohns Disease have an additional risk factor of chronic gastrointestinal inflammation. Would it be prudent to lower this upper limit?

REVIEWER	Lee A. Denson, MD Associate Professor, Pediatrics Cincinnati Childrens Hospital Medical Center United States I have no competing interests with the authors or the manufacturer of the IGF-I used in the study.
REVIEW RETURNED	17-Mar-2013

THE STUDY	There are no supplemental documents.
GENERAL COMMENTS	This is an important, novel, and well executed study. The results are clearly described. The only potential points of clarification are: 1) Was a more direct measure of systemic inflammation, such as CRP or ESR itself, rather than the more global PCDAI, tested in the model? 2) While the simulation study results are encouraging, were the number of children studies (10) truly sufficient to derive a model which would be ready for clinical practice, or is further study in a larger group needed to validate the model prior to use in practice? This could perhaps be more clearly addressed in the Discussion. 3) Similarly, human growth hormone has also been shown to increase IGF-I in pediatric CD. It may be useful to address the scientific and practical pros and cons of further testing of hGH versus hIGF-I in this setting in the Discussion.

Reviewer: Dr Joanne Blair
Consultant Endocrinologist and Honorary Senior Lecturer, Alder Hey
Children's NHS Foundation Trust, Liverpool L12 2AP
I have recruited patients to a study investigating the use of growth hormone in Crohn's
Disease.
I am in discussion with the chief investigator of a study investigating IGF-I in the treatment of
short children with Crohn's Disease. We may also recruit patients to this study.
Please note that I am not qualified to comment on the mathematical methodology in this paper.
It
is important that the paper is also reviewed by an expert in this field.
Thank you very much indeed for giving me the opportunity to review this interesting and well
written paper in which the pharmacokinetics of IGF-I are investigated in eight short children
with
Crohn's Disease, and a mathematical model is presented which facilitates predictable IGF-I
levels following IGF-I administration.
I am not qualified to comment on the mathematical model described in this paper, and I will
therefore limit my comments to the clinical aspects and utility of the model, recognising that
some of these queries may have been addressed in the model. However, to aid the reader
without the mathematical expertise of the authors, it would be helpful if they could be
discussed
more explicitly in the paper if this is so.
Could the authors give more information regarding the reference data from which the IGF-I
SDS
are derived?

These are derived from the work of Esoterix in California. This company was chosen
for our assays, because they have accumulated a large amount of normative data in the
past. This was a major reason for using this company.

IGF-I increases throughout childhood, with marked increases during puberty. In Crohn's
disease puberty is often delayed, and this is the case for some of the patients included
in this study, for example LN08, who is 14.66 years old is prepubertal. If his baseline
IGF-I
SDS was derived from an age related reference data, it would be significantly lower than
if it was compared to a reference set based on pubertal development. Likewise, if the
target IGF-I SDS on IGF-I treatment was derived an age related, rather than a puberty
related reference range, it may give an inappropriately high level for his stage of
development. How have the authors dealt with the effect of puberty / pubertal delay on
IGF-I levels?

Yes, the SDSs were derived from age-related reference data. It is true that IGF-1 increases during
puberty; and to examine this point, we have now recalculated the IGF-1 SDS values based on bone
age from wrist X-rays taken on entering the study. Although not exactly aligned, delay in puberty
correlates well with delay in bone age. Below are the figures that are equivalent to those of 1a in the
manuscript (showing IGF-1 SDS before and after an initial bolus) but calculated according to bone
age,
rather than chronological age. The new figures show, that although differences exist in the details,
using bone age does not change the overall effect. We therefore propose to put these figures into this
response, rather than adding the figures to the text. *We have however addressed this point in a new*
paragraph in the Discussion.
Figure: Increases in IGF-1 SDS with subcutaneous rhIGF-1, based on bone age rather than
chronological ages of the children studied

The low IGFBP-3 levels reported in the study subjects will have two implications: (1) injected
IGF-I will be cleared more rapidly from the circulation than in healthy subjects and

We don't concur with the reviewer that lower IGFBP-3 will result in more rapid clearance. It is now
generally accepted that changes in binding protein concentrations do not alter the clearance of a
protein
bound molecule. This has been an area of interest in pharmacokinetics and is discussed in the
following
article: Benet LZ, Hoener BA. Changes in plasma protein binding have little clinical relevance. Clin.
Pharmacol. Ther. 2002;71:115-121.

(2) the ratio of free (i.e. biologically active) IGF-I: bound IGF-I will be higher in study subjects than in healthy subjects, from whom the reference data are drawn.

This is an interesting point . The assay measures total IGF-1, rather than free concentration so our measurements are both bound and unbound. However, in our study the changes in IGFBP-3 are not great: all subjects were within two standard deviations of normal, even in the most affected child. We therefore do not envisage binding protein changes to radically alter total concentrations in our subjects.

Furthermore, from fundamental pharmacokinetics: the steady-state concentration = dose rate divided by

clearance and average concentration = Area Under the Curve (AUC) divided by time (recall AUC = dose

divided by clearance) . From this it can be seen that the changes in volume of distribution which would

be caused by changes in binding protein should not affect steady-state or average concentrations. These both depend on clearance rather than volume of distribution. For these reasons, we do not believe that correcting for binding protein is necessary in our work; but we agree with the reviewer that

research on assessing free concentrations (using a specific assay) may have value in future pharmacokinetic studies that focus on IGF-1 distribution in the body.

The investigators took alpha-1-antitrypsin to be a marker of protein losing enteropathy, which they speculate would be a marker of IGFBP-3 levels, and therefore investigated the relationship

between stool alpha-1-antitrypsin and IGF-I clearance. They reported no relationship between these two measures. Was there any relationship between IGFBP-3 and IGF-I clearance?

This is a similar point to the point before last. We did not measure the effect of IGFBP-3 on clearance because we do not envisage that the concentrations of binding proteins will affect clearance.

Is it correct that the mathematical model ensures that patients with an IGF-I SDS of <2.5 SD, based on measures of total IGF-I, also have a free IGF-I SD < 2.5 SD?

The reviewer is correct. The model ensures that any free IGF-1 will also be < 2.5 SD.

Finally, could the authors comment on their choice of an upper limit of IGF-I levels of +2.5SD? This upper limit is based on the observation that patients with acromegaly and IGF-I levels >2.5SD for ten years or more have an increased risk of bowel malignancy. Patients with Crohns

Disease have an additional risk factor of chronic gastrointestinal inflammation. Would it be prudent to lower this upper limit?

We agree that this would be prudent. We have changed the parameters to the utility model to accommodate this point. We have lowered the mean of the IGF-1 from +0.75 SD to 0.5 SD.

This results in a lower dosing regimen, and we have changed it in the text. We have redrawn the utility

figure (5), as the means and SDs are lower. We have changed the graph to describe % of children < +2.0 SD rather than < +2,5, with changes in the legend where they are needed.

Reviewer: Lee A. Denson, MD
Associate Professor, Pediatrics
Cincinnati Childrens Hospital Medical Center
United States
I have no competing interests with the authors or the manufacturer of the
IGF-I used in the study.
This is an important, novel, and well executed study. The results are clearly described. The only
potential points of clarification are:
1) Was a more direct measure of systemic inflammation, such as CRP or ESR itself, rather than the more global PCDAI, tested in the model?

Both CRP and ESR were tested independently, but were not significant, whereas the PCDAI and the wPCDAI were. We found this result interesting in that the global disease measure was highly correlated with production rate whereas the blood inflammation markers alone were not. We have too small a sample size to really justify this statement in the text, but it is tempting to conclude that this result provides validation for the composite PCDAI scoring system, over individual blood markers.

2) While the simulation study results are encouraging, were the number of children studies (10) truly sufficient to derive a model which would be ready for clinical practice,

or is further study in a larger group needed to validate the model prior to use in practice? This could perhaps be more clearly addressed in the Discussion.
We agree that the model is not ready for clinical practice. We stated in the limitations section of the Article Summary of the original submission, that further studies are required before this can happen. This model allows us now to undertake those long term studies with an appropriate dose of rhIGF-1. *To emphasize this point, we have adding in the discussion that testing on further subjects is necessary* We have now received funding for a feasibility study into setting up a multicentre randomised controlled study, where the dosing formula can be tested in children.

3) Similarly, human growth hormone has also been shown to increase IGF-I in pediatric CD. It may be useful to address the scientific and practical pros and cons of further testing of hGH versus hIGF-I in this setting in the Discussion.
We agree with this. Although we referenced studies on hGH in the original text [35, and 36], *this point has been strengthened in the Discussion of the resubmission.* Our multi-centre feasibility study aims to compare hGH and rhIGF-1 is a joint award with Professor Faisal Ahmed, who led a study examining the efficacy of hGH in children with Crohn's disease. Although we do not wish to describe the proposed study in depth (because the final protocol is not finalised), *we have stated that future studies should compare IGF-1 with hGH.*

VERSION 2 – REVIEW

REVIEWER	Dr Joanne Blair Consultant Endocrinologist and Honorary Senior Lecturer Alder hey Children's NHS Foundation Trust, Liverpool, United Kingdom I have previously recruited patients with inflammatory bowel disease to a study of growth hormone therapy, and am in discussion with another research group regarding the recruitment of patients to a PK study of IGF-I.
REVIEW RETURNED	23-Apr-2013

THE STUDY	There is great emphasis in this paper on the safety of calculating IGF-I doses using the mathematical model, however the effect of low IGFBP-3 on free IGF-I is not clearly discussed. The low IGFBP-3 concentrations in the face of a high normal IGF-I could increase free IGF-I levels to above the upper limits of normal. This should be considered in the discussion.
GENERAL COMMENTS	Thank you for giving me the opportunity to comment on this revised paper. In my previous review I had two primary concerns: (1) the effect of pubertal delay on the calculation of IGF-I SDS and (2) the safety implications of maintaining IGF-I levels up to 2.5 SD in patients with IGFBP-3 levels between -1 and -2 SD, in whom there is an increased risk of malignant disease of the colon. The authors have addressed these issues in part. They have considered the effect of puberty on IGF-I SD by calculating IGF-I SD from the bone age as well as the chronological age, and demonstrated little effect. With regard to the safety implications of maintaining IGF-I levels at 2.5 SD in this cohort of patients patients with low IGFBP-3 levels and an increased risk of malignant disease of the colon, the authors have revised down the upper limit of the target IGF-I range to 2 SD. I think this is sensible however, I would invite them to comment more explicitly on the issue of free IGF-I. There is great emphasis in the paper on the safety of the reported approach. Population studies have reported that those in whom IGF-I levels are in the upper limits of the normal range, and IGFBP-3 levels are in the lower limit of the normal range are more likely to develop breast and prostate

	malignancy than those in whom the reverse is true. As malignant disease of the colon occurs more commonly in those with high IGF-I levels, it is possible that this association is also true for colonic carcinoma. It may be that this is not relevant in a cohort of patients likely to be exposed to these levels during childhood and adolescence only, but I think it is an omission not to address this important issue.

REVIEWER	Lee A. Denson MD Associate Professor Cincinnati Children's Hospital Medical Center United States
REVIEW RETURNED	25-Apr-2013

- **The reviewer completed the checklist but made no further comments.**

VERSION 2 – AUTHOR RESPONSE

"The reduction in IGFBP-3 raises the question as to whether giving rhIGF-1 to children with a reduced IGFBP-3 may increase free IGF-1 concentrations. Since we did not have access to an assay for free IGF-1, our data are not totally informative on this issue. However, they will not have risen to unsafe levels from saturating the IGF-1 binding capacity. The concentrations of IGFBP-3 are not greatly depressed in the affected children. Even in the most severe case, the IGFBP-3 is within two standard deviations of normal. In addition, IGF-1 is over 95% bound to IGF binding proteins, with an excess of binding capacity. In general when analysing pharmacokinetic data, changes in protein binding do not affect free concentrations but may affect free fraction. This means that for two individuals with the same total concentration, the free concentration maybe elevated in the one with lower binding protein. However, two individuals with different IGFBP-3 concentrations given rhIGF-1 will not achieve the same total concentrations, because as IGF-1 undergoes first-order elimination, higher free concentrations will be more rapidly eliminated due to homeostasis. IGFBP-3 was not a significant covariate for volume of distribution in our model, on analyzing our small dataset. Indeed, our model predicts the majority of patients will have total concentrations just above the normal range."