

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Comparison of three tests for faecal calprotectin in children and young adults: a retrospective monocentric study
AUTHORS	Prell, Christine; Nagel, Dorothea; Freudenberg, Folke; Schwarzer, Andrea; Koletzko, Sibylle

VERSION 1 - REVIEW

REVIEWER	Kaija-Leena Kolho Children's Hospital, University of Helsinki, Finland
REVIEW RETURNED	27-Dec-2013

GENERAL COMMENTS	<p>It is important for the clinicians to realize the variability of the absolute values of fecal calprotectin in different assays. Minor revision needed to further clarify the findings.</p> <p>Minor revision:</p> <ol style="list-style-type: none">1. Introduction: please modify the references as no.2 is not a good choice when characteristics of fecal calprotectin are discussed. You could choose a review here.2. Patients and Methods p. 5: here is stated that patients were excluded if they were younger than 2 years of age. Hence, in results section you need not to clarify the exclusion of the 19 patients <2 years of age. However, if the aim is to compare the performance of three different assays of calprotectin, the age of the patients does not matter. You could exclude such patients only from the more specific analyses when you compare the categorization of the patients according to the given cut-offs.3. Results: Table 3 should be modified. It includes all the information but it is difficult to follow. Add subheadings e.g. Test A versus Test B and give only the data considered as clinically meaningful (e.g. x out of y samples categorized to ...).4. Discussion: p. 10 lines 9-10 use IBD instead of full wording p. 10 lines 49-54 indicate that Aomatsu studied children as endoscopic activity scores and calpro have been compared in adults long before this study p.11 lines 37-38 you may consider adding a paediatric reference including patients with juvenile polyps and high levels of fecal calprotectin (Kolho KL, Raivio T et al Scand J Gastroenterol 2006;41:720-5)
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REVIEWER	Prof Roy Sherwood Clinical Biochemistry Dept King's College Hospital Denmark Hill
REVIEW RETURNED	03-Feb-2014

GENERAL COMMENTS	<p>The authors have carried out a comparison of three different commercially available methods for the measurement of faecal calprotectin. The title is somewhat misleading as it refers to a 'paediatric' population whereas Table one shows that some subjects were over 20 years of age and looking at the age distribution many would have been over 18 which would be considered the upper age for 'children' in most parts of the world. It might be better to refer to the subjects as 'children and young adults'.</p> <p>On page 5 the authors indicate that 'the disease activity...was judged by a paediatric gastroenterologist as clinical remission or active disease', but it is not stated how this was done. Did the gastroenterologist use the PCDAI/PUCDAI scoring system?</p> <p>On page 7 and in the discussion/abstract reference is made to Test C having 'a clear tendency towards higher values'. This is not backed up by any form of statistical test and is difficult to see from the Figure. It would be helpful if the data could also be presented in a Table showing median (IQR).</p> <p>The labelling of the rows/columns in Table 3 would appear to be back to front. In the top two comparisons the totals in the RHS column are the same for both sets which implies that the tests in the rows are the same. As Test A is the common factor between the two sets this is presumably the test making up the rows and Tests B&C the columns although the labels are the other way around.</p> <p>The authors have used mg/kg for the units whereas the common (and SI) unit is ug/g.</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer #1:

1. Introduction: please modify the references as no.2 is not a good choice when characteristics of fecal calprotectin are discussed. You could choose a review here.

Response:

We changed the reference no.2 and chose a review (Tibble JA, Bjarnason I. Non-invasive investigation of inflammatory bowel disease. World J Gastroenterol. 2001 Aug;7(4):460-5.)

2. Patients and Methods p. 5: here is stated that patients were excluded if they were younger than 2 years of age. Hence, in results section you need not to clarify the exclusion of the 19 patients <2 years calprotectin, the age of the patients does not matter. You could exclude such patients only from the more specific analyses when you compare the categorization of the patients according to the given cut-offs.

Response:

Thank you for this correct remark. We changed the result section: "Of 344 samples measured, 21 were excluded because there was insufficient material available for all three tests; another 19 children were younger than 2 of age." Instead we added in p.8: "Ten out of 19 excluded infants and toddlers younger than 2 years had FC values < 100 µg/g and the remaining 9 children showed elevated values > 100 µg/g, again with a clear tendency towards higher values in test C (data not shown)." As it is difficult to classify these children according to the given cut-offs we excluded them from further analyses.

3. Results: Table 3 should be modified. It includes all the information but it is difficult to follow. Add subheadings e.g. Test A versus Test B and give only the data considered as clinically meaningful (e.g. x out of y samples categorized to ...).

Response:

We changed the table in order to better follow it. We added subheadings and inserted a table to better show concordances and discrepancies between the three different tests.

4. Discussion: p. 10 lines 9-10 use IBD instead of full wording p. 10 lines 49-54 indicate that Aomatsu studied children as endoscopic activity scores and calpro have been compared in adults long before this study

Response:

We changed the manuscript accordingly (see page 10).

5. p.11 lines 37-38 you may consider adding a paediatric reference including patients with juvenile polyps and high levels of fecal calprotectin (Kolho KL, Raivio T et al Scand J Gastroenterol 2006;41:720-5)

Response:

We added the above mentioned paediatric reference (see page 11).

Reviewer #2:

1. The authors have carried out a comparison of three different commercially available methods for the measurement of faecal calprotectin. The title is somewhat misleading as it refers to a 'paediatric' population whereas Table one shows that some subjects were over 20 years of age and looking at the age distribution many would have been over 18 which would be considered the upper age for 'children' in most parts of the world. It might be better to refer to the subjects as 'children and young adults'.

Response:

Thank you for pointing this out. We changed the title (see above).

2. On page 5 the authors indicate that 'the disease activity...was judged by a paediatric gastroenterologist as clinical remission or active disease', but it is not stated how this was done. Did the gastroenterologist use the PCDAI/PUCAI scoring system?

Response:

The disease activity was judged based on clinical history and physical examination at the time of visit. We added this information on page 5.

3. The labelling of the rows/columns in Table 3 would appear to be back to front. In the top two comparisons the totals in the RHS column are the same for both sets which implies that the tests in the rows are the same. As Test A is the common factor between the two sets this is presumably the test making up the rows and Tests B&C the columns although the labels are the other way around.

Response:

We changed the table in order to better follow it (see response to reviewer #1).

4. The authors have used mg/kg for the units whereas the common (and SI) unit is $\mu\text{g/g}$.

Response:

We changed the units to $\mu\text{g/g}$.

5. On page 7 and in the discussion/abstract reference is made to Test C having 'a clear tendency towards higher values'. This is not backed up by any form of statistical test and is difficult to see from the Figure. It would be helpful if the data could also be presented in a Table showing median (IQR). There is some discussion of this in the Discussion section but no indication of possible reasons. The authors may wish to consider the abstract below presented in 2012 (Tomkins et al; Gut 2012; 61: A173-A174) in which recovery studies were carried out using three different calprotectin methods. These demonstrated that the PhiCal method (test B in the current paper) had a mean recovery of added calprotectin of 80% whereas the Buhlmann method (test C) had a recovery of 100%. This would result in the results of the Buhlmann method being potentially 25% higher than the other methods.

Response:

Thank you for this important remark. From our perspective you can see this tendency towards higher values in test C not only in Fig. 1, but also in Table 2. Test C classified more healthy controls (group 4) and children with IBD in remission (group 2) as having FC > 100 µg/g (16% and 40%, respectively). This could be clinically meaningful as it would probably lead to unnecessary interventions. Since we are not allowed to cite an abstract we have added a general remark on this issue. "However, it cannot be excluded that the higher values in test C may be the result of a better recovery rate of calprotectin".

6. There are several published papers relating to the efficiency of the commercially available extraction devices used in faecal calprotectin methods, including the Roche kit used in the current study, compared to weighing samples (see Whitehead SJ et al; Ann Clin Biochem 2013; 50: 53-61). These have shown variable percentage recovery including in some sample values as low as 10% of the weighed out result. It is not clear whether the Roche kit behaves consistently between methods and this should be mentioned.

Response:

We appreciate this useful remark. We added to p.12: "Stool samples were extracted separately for test A, B and C from homogenized stool. Commercially available extraction devices used in FC tests, including the Roche kit used in the current study may recover different rates from the same sample {Whitehead, 2013}. Therefore it is not clear whether the used kit behaved consistently between the three tests."