Author's response to reviews

Title: A novel sero-genetic approach determines the community prevalence of celiac disease and informs improved diagnostic pathways

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Author's response to reviews:

12th July, 2013
ImmusanT Inc.
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Ursula D'Souza, PhD
Senior Editor
BMC Medicine

Dear Dr. D'Souza

Re: MS: 2216813729870576

A novel sero-genetic approach determines the community prevalence of celiac disease and informs improved diagnostic pathways

Robert Anderson, Margaret J Henry, Roberta Taylor, Emma L Duncan, Patrick Danoy, Marylia J Costa, Kathryn Addison, Jason A Tye-Din, Mark A Kotowicz,
Further to your email response dated July 8th following our submission of this manuscript, please find our response to Referee 1’s comments. It appeared that Referee 2 has no particular issues to address.

Referee 1:

1) “Up to now in the current clinical practice, the positivity for tTGA of IgA class, found in screening studies in the general population, needs to be confirmed by EmA positivity before making an intestinal biopsy. Positivity for tTGA IgA (especially at a low titer) without EmA is regarded as a false positive result and patients with this antibody pattern do not usually undergo an intestinal biopsy, but they do have a serological follow-up. So, we agree that the proposed protocol with HLA typing is a step forward for excluding immediately CD, but it is undoubtedly more expensive than the previous one and not applicable everywhere. The Authors should underline that such a protocol can work only in countries with a florid economy.”

We appreciate Referee 1 raising the important issue of feasibility and implementation of the diagnostic algorithms described in Table 3 outside Australia. In Table 4 we compare the clinical and financial outcomes of these models when applied to the Australian community cohorts we report here and using publicly available reimbursement schedules from Medicare Australia. It is important to highlight, that the findings we report are based on community cohorts rather than any particular doctor’s clinical practice or patient population. However, we speculate that the community cohorts we studied might reflect patients presenting to practitioners in primary care. To address Referee 1’s point regarding applicability to other countries we have removed the last paragraph in Discussion:

“To address this problem, we modelled the impact of including confirmatory serology and genetic testing before gastroscopy. Inclusion of genetic testing after either TG2 IgA or TG2/DGP IgA/IgG serology reduces gastroscopies and overall costs incurred. Inclusion of confirmatory serology after TG2/DGP IgA/IgG serology has a similar effect and further reduces costs and gastroscopies without appreciably affecting detection rates. Shifting costs from gastroscopy to laboratory testing promises to reduce overall costs and may be more acceptable to patients and be particularly appealing if access to gastroscopy is limited.”

And substituted the following:

“To address this problem, we modelled the practical and financial impact of including confirmatory serology and genetic testing after initial testing and before “definitive” small bowel histology. Inclusion of genetic testing after either TG2 IgA or composite TG2/DGP IgA/IgG serology reduces gastroscopies and overall costs incurred according to the current Medicare Australia reimbursement schedule. Inclusion of confirmatory serology after initial testing with composite
TG2/DGP IgA/IgG serology has a similar effect reducing gastroscopies without appreciably affecting detection rates, and also lowering overall costs according to pricings in Australia. The applicability and financial impact of the diagnostic algorithms is likely to vary between countries and healthcare systems according to local laboratory facilities and costing. However, the community rates of “positive” and “negative” serologies and genetic susceptibility data presented in the current study can be combined with relevant local costs for laboratory assays and procedures to predict which diagnostic pathway might be the most relevant and cost-effective in a particular setting.”

2) The Authors employ a plenty of antibody tests, some of them probably not indispensable for routine use. Are all the tests included in this study (tTGA, tTGA/DGP IgG, DGP IgA, AGG, AGA, EmA) requested in the clinical practice or we can spare some tests also in order to reduce the costs for CD screening? In my opinion we can have good results by using tTGA of IgA class and DGP of IgG class as first step and EmA IgA as confirmatory test in positive cases. DGP of IgA class, AGG, AGA have a very low specificity as reported in literature. The Authors should indicate an antibody flow-chart for practical investigation.

The sero-genetic strategy employed in this study was to “estimate the prevalence of celiac disease at a population level” rather than in a specific patient group. We were prompted to address the utility of various diagnostic algorithms when we observed a surprisingly high rate of false positives and substantial underestimation of celiac disease prevalence based on initial testing with tTG-IgA, the widely recommended initial screening test for celiac disease, tTG IgA (e.g. NICE Guidelines 2009, NIH 2004, ESPGHAN 2012). Furthermore, we identified two biopsy-confirmed cases of celiac disease that were negative for tTG-IgA and EmA. While we agree with Referee 1 that some clinical guidelines have supported tTGA of IgA alone or combined with DGP of IgG class as first step and encouraged the use of “confirmatory” EmA IgA prior to small biopsy biopsy, our findings and those of Walker et al (reference 1) highlight that histology-confirmed cases of celiac disease will be missed if positive EmA IgA is a requirement for further diagnostic work-up. The “World Gastroenterology Organisation Global Guidelines Celiac disease April 2012” (p11) summarizes the widespread view that, “The (serum IgA endomysial antibodies) test is expensive, observer-dependent, and labor-intensive, requiring expert input for correct interpretation. The target antigen has been identified as tissue transglutaminase (transglutaminase 2). IgA endomysial antibody testing is moderately sensitive (around 80%) and highly specific (with close to 100% specificity) for untreated (active) celiac disease.”

In order to clarify that we consider it premature to identify a single diagnostic approach to celiac disease, the following paragraph has been added at the end of the Discussion p13:

“While it would be premature to formally propose a “single” diagnostic flow chart,
the present study highlights the impact of separating initial laboratory testing by serology from a second set of laboratory tests to further increase the likelihood of celiac disease before proceeding to the endoscopic biopsy and histology which is definitive but also the most expensive and intrusive investigation during the diagnostic work-up for celiac disease. Shifting costs from gastroscopy to laboratory testing promises to reduce overall costs and may be more acceptable to patients and be particularly appealing if gastroscopy is difficult to access or even undesirable.”

We have also reworded certain sentences to clarify that it is the prevalence of celiac disease in the community (cohorts) rather patient groups that has been defined using the sero-genetic method described. See Discussion 2nd paragraph, 3rd sentence, “Building upon this observation, we developed a novel method to estimate the prevalence of celiac disease in two large age-stratified, randomly selected community cohorts based on the relative enrichment for HLA-DQ alleles conveying genetic susceptibility to celiac disease in the group of individuals with abnormal celiac disease-specific serology tests”.

Furthermore, based upon Referee 1 comments we felt it important to further emphasise in the text that the initial screening assay we used measuring combined serum IgA and IgG reactivity to both TG2 and DGP (TG2/DGP IgA/IgG) is distinct for the panel of assays testing IgA specific for TG2, and IgG or IgG specific for DGP. In the text, TG2/DGP IgA/IgG is now consistently prefixed with the word “composite”.

With respect to informed consent being obtained, the following sentence has been added at the start of the Ethics section in Methods p4, “Appropriate consent was obtained from all participants of the study”.

Upon rereading there were some instances of poor grammar, letters being omitted, or phrases that might have been worded. We apologies for letting these slip through, and have taken the liberty to correct them.

The US$ conversion rate for the Australian dollar has also been updated p7 in “Costing of diagnostic algorithms”.

All changes in the text have been highlighted in red.

I trust these amendments adequately address the referees’ and your comments. We look forward to your response.

Yours sincerely,

Robert P. Anderson
On behalf of the authors