Author's response to reviews

Title: A Point-of-Care test for facing the burden of undiagnosed celiac disease in the Mediterranean area: a pragmatic design study

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Author's response to reviews: see over
To the Editor
We are submitting the manuscript revised according to what suggested by referees.

Re. Referee 1

# 1st sentence of the first paragraph of the referee’s comments A reasonable premise and conclusion for POCT use in CD setting regards only countries without laboratories facilities we modified the sentence in the Introduction (rows 3-5 page 4) as follows: IgA anti tissue transglutaminase antibodies (IgA-tTG) from commercial enzyme-linked immunosorbent assays (ELISA) utilized for CD testing require a serum sample, need equipped laboratories and are too expensive for countries with poor resources, such as most of those in the Mediterranean area, where patients and families are not able to reach referral centers or centralized laboratories placed faraway and in Conclusions as follows: We believe that the populations amongst which the dissemination of POCT is the most profitable and most "urgent" to implement are in countries with limited resources, above all rural populations where most of the people are not able to reach the referral centres.

We deleted the last sentence However, also in affluent countries, POCT may contribute to bridging the diagnostic gap of celiac disease if used by family Pediatricians and community health personnel.

# With respect to our statements on PoCT “Easy to perform” and “Low cost”:
- The PoCT is easy to perform; it is the interpretation that can be more difficult and therefore according to the aim of the study our conclusion was Our study suggests that factors that can influence the results of a POCT dissemination in the Mediterranean area, apart from the prevalence of CD in different settings where it is applied, include interpretation of results by appropriately trained personnel performing the test.
- With respect to costs, PoCT is cheap because its cost is about 2.2 euro. In comparison tTG is 6-fold more expensive. Moreover, PoCT allows to contemporarily detect IgA deficiency that otherwise should be excluded in order to avoid a false negative of IgA- tTG assay.

# Second paragraph: An ideal diagnostic test should be 100% sensitive and specific, but this rarely occurs. For screening purposes, as those claimed for this POCT, sensitivity must be privileged and should be as high as possible, while a low specificity might be accepted. This does not appears to be the case of this POCT (PPV of about 90% and NPV of about 98%). We agree that a screening test should have a very high sensitivity and as matter of fact this is the case of PoCT considering its high NPV of about 98%

# Third paragraph: The results presented in table 2 are not clearly described. i.e. are the No CD found the number of patients with a confirmed histological diagnosis of CD? As it can be read in the Patients and Method session, Sensitivity, specificity, Positive and Negative Predictive Value (PPV and NPV, respectively) and positive and negative likelihood ratio were calculated for POCT performed in patients referred to the Celiac Center in Italy. Intestinal biopsy was performed in all subjects in whom one of the tests, including POCT, was positive at the Center, and Marsh type 2 (in children) or 3 at histology was considered the gold standard of CD diagnosis. we state that CD diagnosis was made according to the gold standard that is Marsh type 2 or 3
A more detailed analysis of POCT results in comparison with standard ELISA should be presented. In particular was there any association between POCT results and ELISA tTG levels? In the paper by Raivio et al (ref 16) it appears clearly from figure 2 that the lowest ELISA value among those with POCT positive results is about five fold the cut-off. If this is the sensitivity of POCT this must be defined.

We give the answer at page 8 rows 20-22 and page 9 rows 1-2 where we say Out of 20 children with positive tTG confirmed at the Center, 3 had low titers of tTG (< 4 X upper limit of normal) and absent antiendomysial antibodies. According to what requested by families they have not undergone yet duodenal biopsy and are in follow up at a gluten containing diet.

In order to define sensitivity we added: The lowest ELISA value among the 17 children with PoCT positive in whom CD diagnosis was confirmed was about four fold the cut-off.

Some considerations on CD prevalence among the different countries studied should also be taken into account. By POCT CD was identified in Italy in 19/3559, in Slovenia in 7/1480, in Turkey in 1/771. Do these prevalences fit with data in the literature?

Yes they do, as these prevalences fit with data in the literature because that found in Italy ranges (95%CI) between 1:125 and 1:343, in Slovenia between 1:121 and 1:416. One can figure out that increasing the sample the same figure found in other studies can be reached.

Re. Referee 2
We modified the sentence what the factors are that can influence...” into “what are the factors that...”

We shortened the Discussion (246 words) as follows

Pages 10 and 11 we deleted the paragraphs:

However, the high benefit/cost/ ratio to disseminate this POCT in primary care is highlighted by the high per thousand rate of CD yielded by the POCT in this setting. Even though 1 in every 38 children referred to the Centre was false positive, the benefits of finding unrecognized celiac patients widely overcome the cost of the test due to false positive results, both in economic terms such as cost savings, and in terms of costs due to the level of morbidity that CD induces. Therefore in affluent countries, the search for CD should be encouraged at the primary care physician office using this rapid test also considering that in this setting in Italy and Turkey the refusal rate of parents and children was very low.

Page 11 we deleted the following details regarding the methods of the studies performed in Greece and Tunisia

Those who had positive results on the rapid test were referred to a pediatric gastroenterologist for further assessment with serum IgA-tTG and IgA antiendomysial antibodies. In the cases where serum IgA deficiency was identified, serum IgG antiendomysial antibodies and IgG-tTG were subsequently measured. Children with either “positive” or “doubtful” results or with suspected IgA deficiency from the POCT were sent to the referral Celiac Center to perform a complete check-up, including CD conventional serology, i.e. IgA-tTG and antiendomysial antibodies, and total IgA in those with suspected IgA deficiency.