eosinophil counts in assessment of functional bowel disease, but emphasize the role of mast cells in these patients. In these studies, mast cell numbers per high power field were significantly and clinically different from control subjects. Why were mast cells not significantly different in this cohort?

Even though the method of Walker was cited as guidance for studies of biopsies, this paper reviews the work of others and does not comment on the adequacy or validity of the 5-biopsy assessment. Walker was silent on the evaluation of rectal biopsies. What references were used for these studies?

Eosinophil counts in patients were statistically significantly different from control subjects, but not clinically different. It is not clear how a clinician would use a finding of eosinophil counts in a specific patient, as is suggested in the "what you need to know" section of this article.

I agree with the authors that reliable clinical markers are needed to help physicians to better categorize these patients. Clear identification is critical and guides treatment.

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**Conflicts of interest**
The authors disclose no conflicts.

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**Reply.** We would like to thank Professor Weiner for his interest in our study and for his questions.

Regarding the first question, however, we believe that there is a clear answer in the article itself. We stated, in the Methods section, that "the biopsy specimens were assessed in Palermo by 2 pathologists (G.G. and/or A.M.F.)"; the eosinophil count, which seems to be one of the most important findings of the study, "was further assessed by an experienced gastrointestinal pathologist (V.V.) in Brescia." We also added that "all reviewers were blinded to the diet allocation and final diagnosis of each patient." Consequently, the $\kappa$ value provided in the Results section referred to the agreement between the evaluation performed in Palermo and the evaluation performed in Brescia.[1] No further details were given in Supplementary Appendix 2 regarding this point.

As far as the reference method for the eosinophil count is concerned, we thank Professor Weiner for his question and the opportunity given to us to be more precise. We quoted the Walker and Talley[2] report because in this extensive review it clearly was stated that "normal duodenal counts (of eosinophils) are defined as fewer than 10 HPF [high-power fields] in children and 19/5 HPF in adults, in studies based on control values"; to be precise, the review was referring to another study here.[3] The point of interest is that this upper limit (19 per 5 high-power fields) is almost identical to the one indicated in our laboratory: 40 lamina propria eosinophils per 10 high-power fields.

In the rectal biopsy specimens, the upper limit of the reference interval was fewer than 9 lamina propria eosinophils per 5 high-power fields. This was an internal reference for our laboratory and the method was similar to that described by one of the review authors (V.V.) in children,[4] and validated by another author (A.C.) in adults.[5]

In any case, a recent review[6] underlined the problem of the cut-off value of eosinophils in the different gastrointestinal tract segments.

On the topic of the possible role of mast cells in causing the irritable bowel syndrome (IBS)-like symptoms, we found a trend toward higher values in the nonceliac wheat sensitivity (NCWS) patients than in the non-NCWS controls, although the difference was not statistically significant. On the other hand, despite some relevant evidence for a mast cell role in IBS,[7] it has been shown that increased mast cells are not present homogeneously throughout the whole colon mucosa because the increase was detected in the cecum but not in the left colon.[8] Other studies have shown that a significant mast cell infiltration could be detected only in IBS patients with constipation, but not with diarrhea.[9] Furthermore, the relevance of a concomitant presence of eosinophils that, in turn, activate mast cells, has been underlined.[10] Thus, we did not exclude a possible role for mast cells in the pathogenesis of IBS-like symptoms in NCWS patients, but future studies need to be designed to better focus on this point.

Finally, we suggested a possible clinical role for the eosinophil count in NCWS. In fact, as underlined in the Editorial that accompanied our article, our findings showed that "the sensitivity and specificity of the presence of rectal eosinophilia (>9 eosinophils in the rectal lamina propria) was 94% and 70%, respectively, and a positive and negative predictive value for true NCGS of 81% and 89%, respectively."[11] Obviously, this does not mean that an increased number of eosinophils in the rectal mucosa is equivalent to a NCWS diagnosis, but that in the absence of endoscopic findings and/or other obvious causes (ie, parasitic infection), an eosinophil infiltration could be a marker of NCWS and must address the clinician to consider prescribing an elimination diet in subjects with suspected NCWS, as also was confirmed in a recent publication on the matter.[12]

In conclusion, we by no means presume to have found "the diagnostic marker of NCWS," but we think

rather that we have suggested a candidate pathogenetic player (the eosinophil) and a target intestinal site (the rectum) for NCWS disease. These are relevant clinical data to be investigated further in future studies.

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