

Anti-endomysium antibodies detection in the celiac disease screening: Indirect immunofluorescence pattern using umbilical cord sections as substrate

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Summary:

Celiac disease screening test with anti-endomysial antibodies detection is limited by the use of endangered species tissue (monkey distal oesophagus) as well as by the high costs of commercial kits. Due to such limitations new tests are developed to avoid the use of monkey tissue: an IFA test utilising umbilical cord sections (a discarded tissue) and an ELISA test detecting anti-transglutaminase antibodies.

In most cases umbilical cord anti-endomysium test, is not routinely used due to the different pattern of EmA IFA test with monkey oesophagus: in the present article we show some pattern obtained with this new method in order to clarify possible reading misinterpretation.

Zusammenfassung: Nachweis von Anti-Endomysium-Antikörpern bei der Diagnose der Zöliakie: Ein indirekter Immunfluoreszenz-Test unter Verwendung von Nabelschnur-Schnitten Die Diagnose der Zöliakie mit dem Antikörpernachweis auf Endomysium ist durch die Verwendung von Geweben gefährdeter Tierarten (distale Speiseröhren von Affen), aber auch durch die hohen Kosten dieser kommerziellen Nachweis-Kits stark begrenzt. Aus diesen Gründen wurden neue Tests entwickelt, um die Verwendung von Affen-Gewebe zu vermeiden: Es sind dies ein indirekter Fluoreszenz-Test unter Verwendung von Nabelschnur-Schnitten (Abfallprodukten aus der Geburtsklinik) und ein ELISA zum Nachweis von Anti-Transglutaminase Antikörpern.

Meist wird der Nabelschnur-Test nicht routinemäßig angewendet, weil im Vergleich zum Anti-Endomysium-Test mit Affen-Speiseröhren eine unterschiedliche Anfärbung zu beobachten ist. Im vorliegenden Artikel werden einige Färbemuster mit der neuen Methode gezeigt, um mögliche Fehlinterpretationen beim Ablesen der Anfärbungen zu verhindern.

Keywords: celiac disease, endomysium, EmA, umbilical cord, transglutaminase.

1 Introduction

Celiac disease is an inflammatory condition of the small intestine precipitated by the ingestion of wheat in individuals with certain genetic predisposition. The onset of illness most commonly occurs around age two, after wheat has been introduced into the diet, and in early adult life. The only therapy currently known is the gluten free diet to be followed for all the life.

Individuals with celiac disease may experience severe symptoms such as diarrhea, weakness, and weight loss indicating a marked decrease in intestinal absorptive surface area involving much of the small intestine. Other extraintestinal manifestations of celiac disease include osteopenic bone disease, tetany and rarely neurologic disorders. Removal of wheat (gluten) from the diet of individuals with celiac disease or gluten sensitivity results

in regeneration of the intestinal mucosal absorptive surface area and resolution of symptoms in most patients.

This disease can cause consistent isthologic damages and functional insufficiency if not diagnosed in time; therefore, several screening tests have been introduced to find out the entire coeliac subject in the paediatric age. The most utilized diagnostic methods in clinical laboratories are IgA and IgG anti-gliadin antibodies (AGA IgA and AGA IgG) ELISA test and IgA anti-endomysium (EmA) using indirect immunofluorescence (IFA).

The two type of test have advantages and disadvantages:

- EmA IFA tests are highly specific (100%) and sensitive (96-98,5%) while AGA ELISA are high sensitive with a low specificity.
- EmA tests utilize monkey distal oesophagus sections, a protected species that is killed for the use of its tissue.

In order to avoid the use of monkey oesophagus two new diagnostic methods have developed:

- EmA IFA test using umbilical cord sections (HUC);
- ELISA test using transglutaminase as substrate.

2 EmA IFA test using umbilical cord sections (HUC)

The EmA IFA test with umbilical cord sections as substrate is a simple, specific (100%) and sensitive (98.5%) method whose performances overlaps the IFA test with monkey oesophagus.

The great advantage of the test is to be connected with the possibility to avoid the use of monkey's tissue, a protected species which must be killed to use the oesophagus for diagnostic purposes. Moreover, the use of umbilical cord permits a

price reduction of at least 40% of the diagnostic EmA IFA kit.

Since 1995, a great number of scientific papers demonstrate the high performance of the HUC IFA test. The most important are Volta et al. (1995), Volta et al. (1996), Pittchleir and Ladinser (1996) and Kolho and Savilathi (1997).

The new HUC IFA test, in most cases, is not routinely used due to the different pattern of EmA IFA test with monkey oesophagus. With the new test it is necessary to completely change the sight of results.

This is a short guideline to help in the orientation of the new patterns, when wells are read at the microscope

a- sections show three reactive areas: two arteries and one vein;

b- IgA anti-endomysium reacts with the endomysial fibres surrounding the vessels and also close to the endothelium;

c- the tissue outside the vessels have not be considered for the results;

d- positive results show green vessels, negative results show red vessels (due to Evans blue as counterstain).

e- sections should be read with 10X, 25X and 40X oculars.

The figures show that the use of HUC IFA test is a very simple diagnostic tool to detect celiac disease subjects; the obtained patterns are very simple to identify. The test procedure and performances are quite identical or better to those of monkey oesophagus IFA test.

For the sum of these reasons, the HUC IFA test should be known by the majority of lab practitioners and celiac disease scholars.

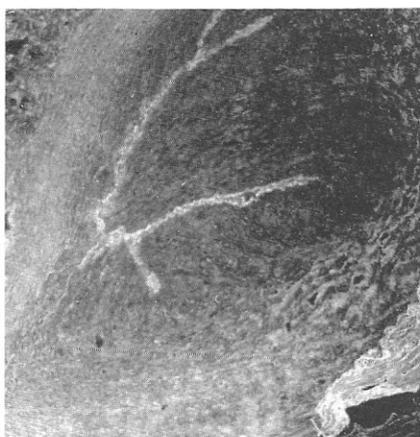


Figure 1: Umbilical cord artery: high positive (+++) result in the endomysium surrounding vessel and close to endothelium. f 20X.

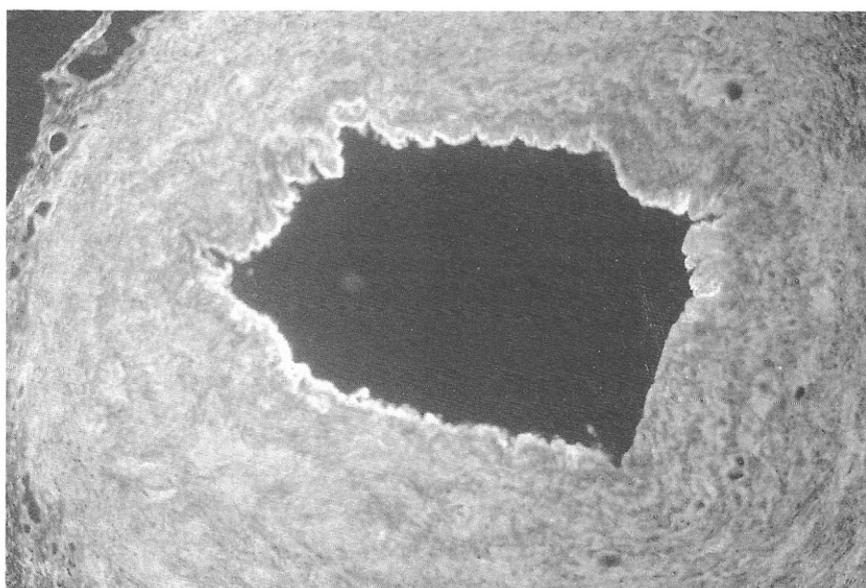


Figure 2: Umbilical cord vein: high positive (+++) result in the endomysium surrounding vessel. f 20X.

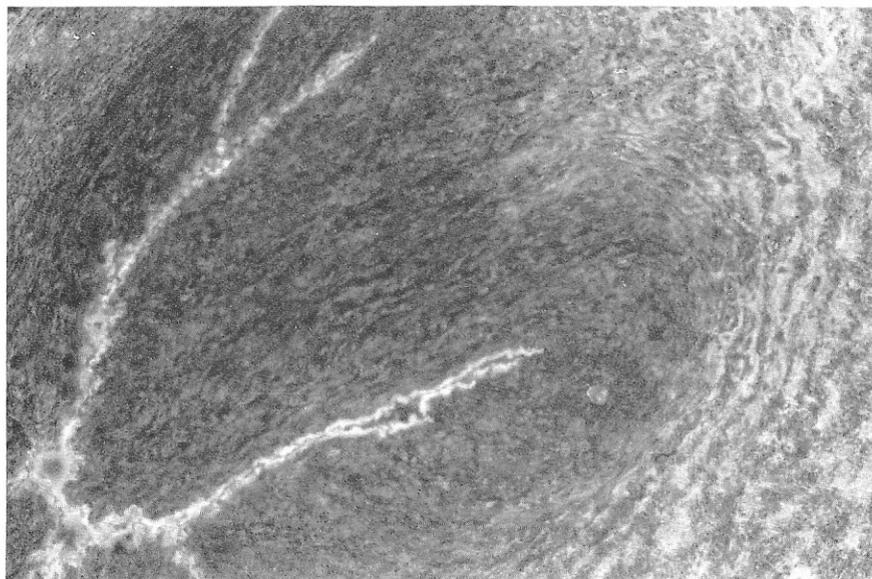


Figure 3: Umbilical cord artery: particular of figure 1. f 40X.

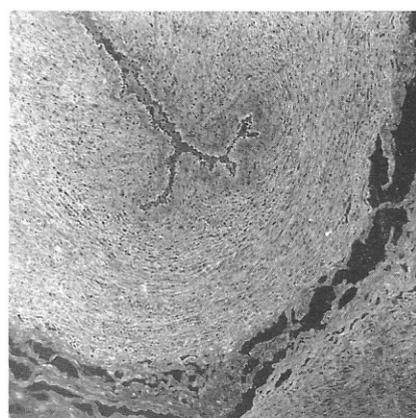


Figure 4: Umbilical cord artery: high positive (+++) result. f 10X

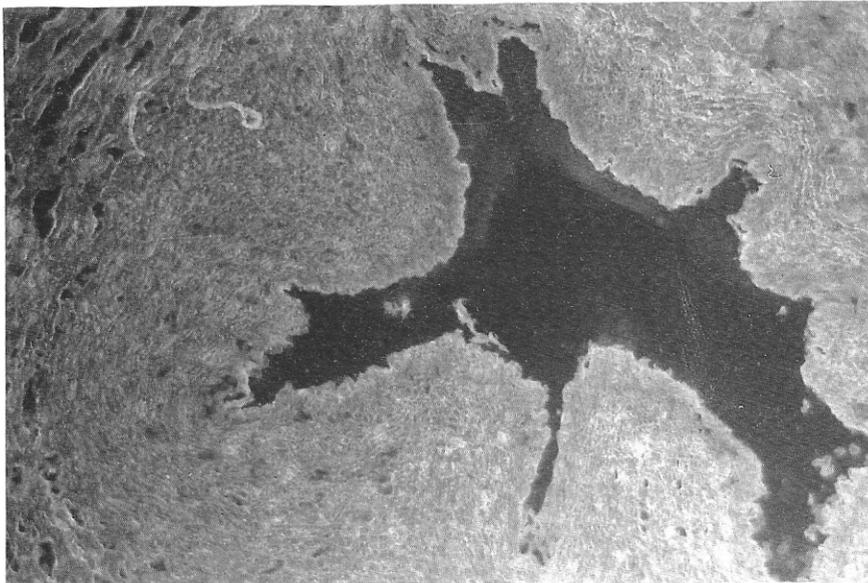


Figure 5: Umbilical cord vein: positive (++) result in the endomysium surrounding vessel. f 40X.

3 ELISA test using transglutaminase as substrate.

Tissue transglutaminase (tTG) is a calcium dependent transamidating enzyme that catalyze protein crosslinking resulting in the formation of an e-(g-glutamyl)-lysine bond and gliadin is one of the substrate for the enzyme. In the past, various scientific articles defined a strict correlation between the intestinal mucosa tTG and celiac disease.

Recently, some authors described the tTG as the autoantigen of the celiac disease (Dieterich et al., 1997 and Maki, 1997) or, in other words, the endomysial unknown antigen: this important discovery could permits to change the EMA IFA tests into EMA ELISA test reappointed now anti-tTG ELISA test.

In order to evaluate such a possibility, Authors developed an anti-tTG ELISA test and compared results obtained utilising celiac, pathological and healthy subjects sera, with an EmA IFA test on distal monkey oesophagus and on umbilical cord sections. Our first results shows a complete overlapping between the tTG ELISA and EmA IFA test.

The new finding must be confirmed with a large scale screening but if results will confirm our first data, is reasonable to suppose that such a test will substitute those in the IFA methodology, helping in the process of total elimination of any ani-

mal part contained in diagnostics useful for the detection of celiac disease.

In conclusion, we can affirm that nowadays times are mature enough to allow the substitution of diagnostics with monkey oesophagus with those containing discarded tissues (umbilical cords). Furthermore, in the near future any tissue will be eliminated with the use of the diagnostics in the ELISA methodology for the detection of IgA anti-transglutaminase antibodies.

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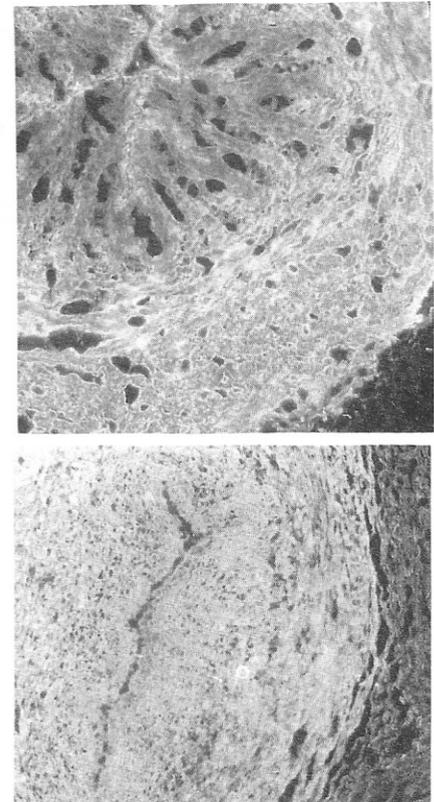


Figure 6 and 7: Umbilical cord arteries: negative results. f 20X

Anti-endomysial antibodies on human umbilical cord tissue for celiac disease screening. Save both money and monkeys. *Dig. Dis. Sci.* 40,9, 1902-1905.

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