ACTIVITY A.T3.2 IMPLEMENTATION OF PILOT PROJECT

D.T3.2.5 IMPROVEMENT OF DIAGNOSTICS

1. RESULTS ACHIEVED ACCORDINGLY TO OBJECTIVES

- **Please review the objectives you have set up in your D.T3.1.1 description, in the Status report Phase 1 and describe activities and results achieved by your pilot. Give an overview of the processes that are part of your pilot project.**

The pilot project of PP4 (Deliverable D.T3.2.5) is titled “Improvement of diagnostics, with testing 3 methods to improve CD practice”.

The aim of our first proposal of the pilot project was to determine the implication of fecal calprotectin, fecal alpha-1-antitrypsin and fecal elastase-1 in the diagnosis, follow-up and response to treatment in pediatric celiac disease patients. With the inside of medical literature published after our first pilot project proposal, and because of technical and administrative problems, with consensus of other pilot project partners and with permission of JC, we gave up from our first pilot project proposal. We changed one biomarker: instead of fecal alpha-1-antitrypsin, we measured gluten immunogenic peptides in stool samples.

Our study was conducted to compare fecal calprotectin, fecal elastase-1 and gluten immunogenic peptides levels in 22 untreated (i.e. newly diagnosed) CD patients with 45 children with previously diagnosed celiac disease on a gluten free diet and with a control group of 57 healthy children.

As fecal calprotectin is a granulocyte cytosolic protein considered to be a promising marker of subclinical inflammation of the gastrointestinal tract, the idea was to correlate fecal calprotectin levels with the inflammation of the gastrointestinal tract in CD in three defined groups.

The study found no statistically significant difference between FC levels in newly diagnosed CD patients, those on gluten free diet and controls.

We have shown no comparison between FC levels of 22 untreated paediatric CD patients and control group of 57 healthy subject.

According to our study, there is no evidence to support the use of FC as a biomarker of subclinical inflammation in CD, i.e. the use of FC as a diagnostic biomarker for CD in the paediatric population. Randomly measured FC levels in CD patients are within normal levels, and elevation of FC should not be explained by CD and clinician should do the further investigation for inflammatory bowel disease.

Considering protein loss in celiac disease, as a result of breakdown of enterocyte barrier with villous atrophy, we were assuming that the measuring of fecal alpha-1-antitrypsin could be used for the estimation of enteric protein loss in CD patients.

In 24% (11/46) patients with previously diagnosed CD, we found decreased levels of fecal elastase-1. In healthy children we also found decreased levels of fecal elastase-1 in 21% (12/56) subjects. According to these small sample size data, fecal elastase-1 is unspecific biomarker and it is not helpful in diagnosis, follow-up and response to treatment in paediatric celiac disease patients.

Detection of gluten immunogenic peptides in faeces have been proposed as new non-invasive biomarkers to detect gluten intake and to verify gluten free diet compliance in CD patients. Gluten immunogenic peptides identify voluntary or unknown consumption of gluten within 48 to 72 hours after the transgression.

The gluten immunogenic peptides test in faeces (by ELISA) was positive in 20/22 samples of the newly diagnosed patients and in 25% (14/56) previously diagnosed CD patients who should follow gluten free diet. These results showed that every 4th CD patient is not strictly adherent to the gluten free diet. Two negative results in first group of patients should raise awareness, to the patients and to the physicians, that patients must me on gluten containing diet in the time serology tests are done, to avoid the false negative results.

2. ADDED VALUE OF THE DEVELOPED & TESTED PILOT SOLUTION IN YOUR REGIONAL ENVIRONMENT

- Please describe shortly, what is the gained added value for the end-user of pilot service solution

ADDED VALUE for END-USER
Short term effects
1. To determine the implication of fecal calprotectin, fecal elastase-1 and gluten immunogenic peptides in the diagnosis, follow-up and response to treatment in celiac disease patients
2. To establish if this non-invasive biomarkers are reliable enough to implement them in everyday practice
3.

3. DEVIATION AND PROBLEMS ENCOUNTERED

- **In case your outcomes are different from the planned, please give an explanation of the reasons and formulate your modified results achieved. Was your planned model working or did you had to make modifications, if yes, describe? Did you had any problems in you pilot implementation? If yes, which was the solution adopted?**

- With the inside into the medical literature published after pilot project proposal in AF, and because of the constructive critics of other project partners we gave up from our initial pilot project proposal. Instead of measurement of of fecal calprotectin, fecal alpha-1- antitrypsin and fecal elastase-1 in stool sampels of newly diagnosed CD patients, patients with already known CD and

control group, we measured calprotectin, fecal elastase-1 and gluten immunogenic peptides (GIP) in stool samples.

- Since we had no possibility to test stool samples for GIP, partners from Italy tested all stool samples for GIP.
- We had no enough newly diagnosed CD patients in examined period, so we analyzed stool samples of newly diagnosed patients from Italy.

4. LESSON LEARNED RELATED TO CO-CREATION OF PILOT SOLUTIONS WITH ENGAGED STAKEHOLDERS

- Please describe what were the benefits and setbacks related to co-creation of pilot project with stakeholders.

LESSONS LEARNED
Benefits
1.easiness of collecting materials
2.parents' wiliness for giving the samples
3.

5. FURTHER ACTION PLAN (ACTIVITIES FOR THE FUTURE)

- What are your further activities of the pilot project development,
  - On the local level ?
  - On transnational level ?
- How did you plan to ensure sustainability to your pilot? Have you plan any action for the maintenance/follow up/development of the actions implemented, after the project ends?
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- We wanted to see whether can non invasive way be used in children with newly diagnosed CD and children already having CD. We tried to find can certain biomarkers in stool be used instead of invasive blood sampling. The idea was to try to give our results as proposal for CD diagnosis and follow up non only to local level, but also to national and even international level. Results found in literature were promising enough to start our pilot project. Unfortunately, the results were negative in two out of three conditions and didn't prove their use in everyday practice. The reason for this was maybe in shortness of time for collecting bigger groups of patients; maybe on bigger

sample the results could be different. Anyway, we cannot give any recommendation for the continuation of the fecal elastase 1 and calprotectin biomarkers' usage in everyday clinical practice. As the results weren't positive, we won't continue with using neither of the two biomarkers.

- The only one biomarker that showed usefulness is gluten immunogenic peptides for detecting low adherence to gluten free diet. It can be useful in children ad non-invasive follow up as it is simple to collect and very well follows gluten ingestion. There are more studies needed to see what is the time distance of detecting GIP in stool and serological biomarkers to start to raise and to establish the right time interval for stool analysis. We are interested to see whether can our laboratory start to detect GIP in stool as we are willing to continue with the GIP detection in bigger sample and longer time period.