Listeriolysin O antibodies detection in pregnant women: results from an Italian pilot study

M. Massacesi 1, N. Oliva 1, L. Pucci 2, G. Brandi 3, M. De Santi 3

Key words: Listeriosis, pregnancy, Listeriolysin O, seroprevalence, nutritional habits
Parole chiave: Listeriosi, gravidanza, Listeriolisina O, sieroprevalenza, abitudini nutrizionali

Abstract

Background. Listeriosis is a rare infection affecting primarily pregnant women, the elderly and individuals with a weakened immune system and is caused by the ubiquitous bacterium Listeria monocytogenes. Infection during pregnancy can cause severe consequences especially for the fetus, leading to sepsis, premature delivery, stillbirth and miscarriage.

Study design. A pilot observational study has been conducted in order to establish the prevalence of seroconversion of specific antibodies against a peculiar toxin belonging to L. monocytogenes, listeriolysin O (LLO), in a population of pregnant women from Senigallia (Central Italy) and to find correlations between anti-LLO antibodies seropositivity and health and nutritional information. A total of 60 women were screened for anti-LLO antibody positivity and interviewed during their pregnancies. Statistical analyses were performed to evaluate antibody prevalence in serum samples and potential risk factors.

Results. The seroprevalence resulted 18% (95% CI, 8.2 – 27.7%), corresponding to 11 pregnant women. Categorical principal component analysis and hierarchical cluster analysis revealed a significant correlation between anti-LLO positivity and gastrointestinal pain events and vomit, fever and diarrhea episodes, and a possible association with consumption of pre-cooked meal. No significant correlation was observed in women with a previous miscarriage or with miscarriage cases in their families.

Conclusions. Findings from this pilot study will be used to design a wider study focused on the prevalence of Listeria-specific antibodies in pregnant women and could allow to the identification of nutritional and behavioral habits related to Listeria infection which could lead to significant clinical implications.

Introduction

Listeriosis is a foodborne illness of significant public health concern, caused by consumption of food contaminated with Listeria monocytogenes, a ubiquitous gram-positive bacterium described for the first time by Murray and colleagues in 1926 (1). Listeria monocytogenes includes thirteen serotypes, but only four of them, 1/2a, 1/2b, 1/2c and 4b, are commonly isolated from humans (2). L. monocytogenes is an intracellular pathogen that affects primarily pregnant women and their newborns, the elderly and also the immunocompromised patients (3). Clinical listeriosis is relatively rare but the majority of its cases is associated with hospitalization and with a high case-to-death rate, reaching up 20-30% and, for risk group

1 Principe di Piemonte Hospital, Senigallia (AN), Italy
2 Diatheva SRL, Cartoceto (PU), Italy
3 University of Urbino Carlo Bo, Biomolecular Sciences Department, Hygiene Unit, Urbino (PU), Italy
Listeriolysin O antibodies detection in pregnant women

patients, even 75% (2). It shows a wide spectrum of clinical features, ranging from a noninvasive gastroenteritis to life-threatening sepsis and severe meningo-encephalitis in infants and adults. Infection during pregnancy can cause severe consequences especially for the fetus, leading to sepsis, premature delivery, stillbirth and miscarriage (3).

An early and reliable diagnosis of a \textit{L. monocytogenes} infection is still challenging, due to several reasons: blood cultures could result negative, due to the inconsistent presence of \textit{L. monocytogenes} in blood; vaginal swabs and stool cultures are not recommended due to their low sensitivity and specificity and to the existence of healthy carriers; serological tests have been demonstrated as unreliable due to the host low production of antibodies against membrane and flagellar antigens of this intracellular pathogen (4).

However, the poor and quite aspecific symptomatology of \textit{L. monocytogenes} infection suggests the need for a serological test in order to evaluate the immunological status and detect the disease onset as early as possible. In this context, the presence of antibodies against a specific toxin produced by the bacterium, listeriolysin O (LLO), has been proposed as a marker of infection (5, 6) because it is released in the blood by the pathogen during the early stages of the infection and induces a strong immune response by the host. The immunoglobulins produced by the host belong to serotype G, while IgMs seem to be inconsistently produced. Anti-LLO IgGs are detected after few days from the infection, but do last for months (4).

A commercial kit for the detection of antibodies against LLO is produced by Diatheva (Italy). To date, it is the only available commercially kit, marked IVD-CE and supported by widely recognized scientific research (4, 7).

Prevalence data about anti-listeria antibodies in the population have been previously investigated by several groups, even if referred to limited geographic areas; data have been published on the prevalence among healthy individuals in Portugal (8) and among pregnant women in Iran (9), in Spain (10) and in Turkey (11). Seropositivity resulted 17.1%, 17.5%, 12% and 21.5%, respectively. These data suggest that seropositivity for antibodies against \textit{Listeria} seems to be quite widespread and is consistent with the great diffusion of \textit{L. monocytogenes} in the environment. Nevertheless, in the mentioned studies, anti-\textit{L. monocytogenes} antibodies (and not anti-LLO) were detected by different methods.

To our knowledge, there are no recent studies about seroprevalence of antibodies against \textit{L. monocytogenes}, especially in Italy.

Here, a pilot observational study has been conducted in order to establish the prevalence of seroconversion of specific antibodies against LLO in a population of pregnant women from Senigallia (Marche Region in Central Italy). Moreover, nutritional habits and personal information such as gynaecological history were collected and analyzed. We evaluated the effectiveness of the analysis for the presence of antibodies as a diagnostic aid in the diagnosis of listeriosis in order to clarify whether or not it could be considered as a valid support for the clinician, and to find correlations between anti-LLO antibodies seropositivity and health and nutritional information regarding pregnant women.

Materials and Methods

\textbf{Study design}

The study was approved by the Ethics Committee of The University of Urbino, (Italy) in May 2016. This cross-sectional project involved a population of pregnant women from Senigallia (Italy), who have attended the “Principe di Piemonte” Hospital between December 2016 and September
2017. In the same period, the hospital recorded a total of 532 deliveries. Pregnant women were informed on the observational nature of the study and those who agreed signed the consent form. A total of 65 women were enrolled but 5 were excluded due to incomplete information.

A total of 60 women (mean ± SD age: 33.7 ± 5.1 years) with a median gestational age of 39 weeks (21-41) were included in the study. Personal information regarding the number of previous pregnancies, miscarriages and miscarriage events in family were collected (Table 1). Enrolled women have been grouped into two categories related to their job type. We considered jobs with no potential risk of listeriosis infection and jobs which may increase - even if slightly - the chance to come in contact with the bacterium, as healthcare providers or food handling workers.

Enrolled women were interviewed twice during their pregnancy about nutritional habits (i.e. soft cheese, fresh vegetables, pre-cooked meal and poor cooked meat consumption), antibiotic use, job type and events of gastrointestinal pain, vomiting, fever and diarrhea. For more details about the questionnaire see supplementary material S1.

At the same time, blood specimens were collected and immediately coagulated for sera collection. The samples were tested within 24 hours from collection and stored at -80°C for further analysis. Anti LLO-antibodies were detected using a commercial ELISA assay (Human Anti-LLO IgG Immunoassay, Diatheva) according to the manufacturer’s instructions. Seroprevalence of *L. monocytogenes* among the study population was calculated as the number of serologically positive subjects among the study group.

**Statistical analysis**

Correlations between anti-LLO-positive women and nutritional habits and personal information were evaluated using the categorical principal component analysis (CATPCA) – that allows to analyze both categorical and continuous variables simultaneously – and hierarchical clustering. The categorical variables were converted into numbers and considered as ordinal variables;
in details, “antibiotics”, “gastrointestinal pain” and “vomiting, fever, diarrhea”, were coded as no=0, yes=1; variables regarding food consumption were coded as 0=never, 1=rarely, 2=sometimes, 3=often; LLO-negative=0, LLO-positive=1. Hierarchical cluster analysis was performed by Ward’s minimum variance agglomeration method applied to squared euclidean distances between variables. Statistical analysis was done using the statistical package SPSS (version 17; SPSS Inch., Chicago, IL, USA).

Results

The prevalence of anti-LLO specific antibodies was found to be 18% (95% CI, 8.2 – 27.7%), corresponding to 11 women. Although listeriosis has not been confirmed for any of them, four women received antibiotic therapy during their pregnancy. Table 1 shows the characteristics of women enrolled in the study. Information has been collected and refers to the total of enrolled women and to the seropositive and the seronegative groups. No significant correlation with the presence of anti-LLO specific antibodies was observed in women with a previous miscarriage or with miscarriage cases in their families. Moreover, there was no association between anti-LLO-positivity and the age of mother, weight of the newborn as well as the number of previous pregnancies. A total of 4 women (6.7%) were found to have risky jobs, however no correlation with the presence of the anti-LLO antibodies was highlighted.

On the other hand, positive women reported incidents of fever and/or intestinal pains during pregnancy more frequently than negative ones. Particularly, 45.5% of anti-LLO positive women presented intestinal pains and 27.3% fever and/or vomit (14.6% and 18.8% in negative women, respectively). Regarding nutritional habits, a slight association with pre-cooked food and soft cheese consumption was found: LLO-positive and LLO-negative women declared

![Figure 1](image-url)

**Figure 1 - A.** Categorical principal component analysis of variables (CATPCA). The component map allows to visualize the cluster of correlated variables in groups. Dimension 1 accounts for 18.662% of variance and Dimension 2 accounts for 15.448% of variance. LLO-positive women show a significant correlation with gastrointestinal pain and with vomiting, fever and diarrhea events (red cluster) and a slight correlation with pre-cooked meal consumption (blue cluster). **B.** Ward’s minimum variance cluster analysis. The dendrogram shows the distance between variables using average linkage. LLO-positive women cluster with gastrointestinal pain and with vomiting, fever and diarrhea events.
a “often” consumption of pre-cooked food of 18.2 and 6.3 %, respectively, and a “often” consumption of soft cheese of 63.6 and 45.8 %, respectively.

In order to further assess the correlations among LLO antibody and the analyzed variables, CATPCA and hierarchical cluster analyses have been conducted. CATPCA revealed a strong correlation between newborn weight and gestational age, as expected (Fig. 1). Moreover, CATPCA revealed that anti-LLO positive women correlate with episodes of gastrointestinal pain and episodes of vomiting, fever, diarrhea (Fig. 1A, red circle), and consumption of pre-cooked meal (Fig. 1A, blue circle).

The correlations between anti-LLO positive women and events of gastrointestinal pain and vomit, fever and diarrhea were analyzed by Ward’s minimum variance cluster analysis. The results, shown in figure 1B, confirmed the clusterization of anti-LLO positive women with gastrointestinal events.

**Discussion**

Listeriosis may be a very serious problem during pregnancy, but an early maternal suspicion, refusal of food at risk, diagnosis and treatment may reduce the event of transplacental transmission (3, 12). A timely diagnosis can only be achieved with serological screening of high-risk patients, even though it still is not possible to distinguish between current or prior infection. The detection of Listeria-specific IgG indeed cannot be considered a clear signal of acute listeriosis, because antibodies could have been formed during a past infection (4).

Given the risk of infection during pregnancy (13), the wide and probably underestimated spread of the bacterium and the difficulties of a reliable diagnosis (14), we organized this pilot study in order to identify and evaluate the clinical usefulness of the test for the detection of anti-LLO antibodies from blood as a screening test.

Our results show that a 18% positivity rate can be expected, suggesting that seropositivity for antibodies against *Listeria* is quite widespread. Moreover, we demonstrated a significant correlation between anti-LLO- positivity and events of gastrointestinal pain and a possible association with episodes of vomiting, fever, diarrhea and consumption of little cooked meal and of soft cheese.

These variables seem to be related to the presence of anti-LLO specific antibodies and could be useful to clinicians in interpreting the serological results.

Despite the lack of a rapid and reliable method for the diagnosis of listeriosis among subjects at higher risks of infections, results from this pilot study lead to the conclusion that the screening for the presence of anti-LLO antibodies is not recommended for all pregnant women, especially if asymptomatic because of the high prevalence of seropositivity among pregnant women. The sole detection of specific antibodies without symptoms could give positive results in the case of prior and solved infections, and this would generate unmotivated anxiety and concern in pregnant women and their clinicians. Furthermore, the gynecologists would find difficulties in the management of positive results which cannot be quickly confirmed or denied.

Contrariwise, this test could be of pivotal importance in cases of women showing symptoms or reporting high-risk behaviours (e.g. ingestion of contaminated or recalled food), even if they have already initiated an antibiotic therapy as a precautionary measure, because the presence of specific antibodies is not affected by drugs, unlike blood cultures.

If the infection was actually ongoing, the risks incurred by the patients and their fetuses would be significantly greater and would undoubtedly justify any difficulties in
Listeriolysin O antibodies detection in pregnant women

the clinical management of the results. Only
the suspicion of an infection derived from
an anti-LLO positive result, together with
generic symptoms, could allow the start of
an early and effective therapy.

Any clinical results should be interpreted
and managed by appropriately prepared
gynecologists or obstetricians. The knowledge
of the symptoms, diagnosis and therapy of
listeriosis infections in pregnant women
plays a crucial role in estimating the burden
of the disease and in the evaluation of the
effectiveness of the public health protection
policies, including screening programmes and
clinical management (15).

In conclusion, the detection of anti-LLO
antibodies could represent a useful tool for
the serological screening of people at high
risk of L. monocytogenes infection, when
symptoms are shown.

There is a broad agreement about the
need to inform and adequately sensitize
the healthcare providers involved in the
care of pregnant women (16, 17), and this
improvement would be helpful also for
overcoming the difficulties in managing
the positive outcomes of the test. A deeper
knowledge about listeriosis could also
lead to the standardization of the clinical
evaluation criteria especially in high-risk
situations, reducing the existing discrepancy
among their approaches.

Moreover, findings from this pilot study
will be used to design a wider study, focused on
the prevalence of Listeria-specific antibodies
in pregnant women and could allow to the
identification of nutritional and behavioral
habits related to Listeria infection which could
lead to significant clinical implications.

References

1. Murray EGD, Webb RA, Swann BR. A disease
   of rabbits characterised by a large mononuclear
   leucocytosis. J Pathol Bacteriol 1926; 29: 407-
   39.
2. Chlebicz A, Slizewska K. Campylobacteriosis,
salmonellosis, yersiniosis and listeriosis as
   Zoonotic foodborne diseases: a review. Int J
3. Mateus T, Silva J, Maia RL, Teixeira P. Listerio-
   sis during pregnancy: a public health concern.
   ISRN Obstet Gynecol 2013: ID 851712.
4. Pucci L, Massacesi M, Liuzzi G. Clinical man-
   agement of women with listeriosis risk during
   pregnancy: a review of national guidelines.


Corresponding author: Prof. Giorgio Brandi, University of Urbino Carlo Bo, Biomolecular Sciences Department, Toxicological, Hygiene and Environmental Sciences Unit, Via S. Chiara 27 61029 Urbino (PU), Italy e-mail: giorgio.brandi@uniurb.it