

Prevention of congenital Chagas disease by screening of mothers and monitoring of serological tests of neonates: the seven years' experience

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SUMMARY

Approximately 14000 immigrants coming from the Cochabamba area of Bolivia, with an increased risk of congenital Chagas Disease (CD), are currently living in Bergamo, Italy. According to the World Health Organization (WHO) recommendation (2011), prevention of congenital CD involves testing all pregnant women at risk of infection and performing follow-up of their newborns.

In our study, all pregnant women of Latin American origin were tested for the presence of *Trypanosoma cruzi* antibodies and children, born to mothers found to be positive, were followed up after delivery.

T. cruzi antibodies were detected using a chemiluminescence immunoassay. The test was also performed on siblings and fathers of children with CD, and women of childbearing age to prevent the congenital infection, as proposed by 2011 WHO recommendation.

In the study period 1105 patients were tested for CD, using a serological test: 934 (85%) were females and 171

(15%) were males. Of the 62 newborns, from mothers who tested positive, 28 were females and 34 were males. The number of positive adults and siblings identified was 148 (14%).

Among the adults and siblings born between 1991 and 2011 only 3 (2%) of females tested positive to serological test.

All neonates, with the exception of one, were classified as non-infected according to the follow-up of index value of CD serology.

This study confirms the usefulness of serological tests and of their index value as follow-up.

The difference of positivity rate for CD antibodies between people born before and after 1990 should be further investigated to generate information that potentially improve the prevention and control of CD.

Keywords: *Trypanosoma cruzi*, Chagas disease, congenital, serology, Cut Off Index.

INTRODUCTION

Congenital Chagas Disease may occur in any part of the world and the lack of well-established surveillance programs means that the diag-

nosis is largely missed. Up to 2019, identification of all affected children involved testing all pregnant women at risk of infection, that are either living in an endemic area or having migrated from an endemic area [1]. All children born to seropositive mothers should be tested not only within the first month of life but also at ~6 and ~12 months of age. The diagnosis is made by identification of the parasite using standardized micro-methods before 6 months and by a positive serological test

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after 10 months of age. A one-year follow up period is essential, as a significant proportion of cases are initially negative and are detected only at a later stage by either detection of blood parasites or by seroconversion. The success of the follow up depends on establishing good follow up routines in primary care settings and on extensive counseling of the mothers emphasizing the relevance of control, even in asymptomatic and apparently healthy children [2-4].

Within the first year of life, early diagnosis is essential for effective and well-tolerated treatment [4, 5]. Siblings of children with congenital infection should also be studied. In their first report on neglected diseases (2005) the WHO recognized that the movement of CD to areas previously considered not endemic, resulting from increasing population mobility between Latin America and the rest of the world, represents a serious public-health challenge [6].

It is estimated that 6-7 million people worldwide are infected with *Trypanosoma cruzi*; the highest number of infections is recorded in Latin America, endemic area. As a result of migratory flows, currently most of the infected people live in urban contexts and, increasingly, move to non-endemic countries such as Italy (estimated 9000 to 10000 cases) [7].

Between 3268 and 5015 *T. cruzi* infected subjects are estimated to live in Italy, predominantly in the North-Western Regions [8].

In the study published by Angheben et al. in 2011, it was estimated that in Bergamo province there were about 14000 immigrants coming from Cochabamba area in Bolivia. It was confirmed that among the Bolivians immigrants 30.7% had a positive serological result, which is in accordance with other published studies but in contrast with to Antinori et al., that showed a lower prevalence of positive results [6, 7, 9, 10].

The aim of this paper is to present the results of a project designed to monitor and prevent Congenital Chagas Disease with particular emphasis on the data acquired by the serological tests.

This study was performed according to the World Health Organization (WHO) recommendation published in 2011, before the WHO shifted its focus towards active screening of girls and women of childbearing age to detect the presence of *T. cruzi*, the causative parasite of Chagas disease [5, 11].

Recent publications demonstrate that the diagno-

sis and treatment of women in this age group before pregnancy can effectively prevent congenital transmission [12, 13].

Up to the new 2019 WHO recommendations and according to the 2011 recommendations, control and prevention strategies for Chagas Disease largely improved early detection and treatment of infected neonates and pregnant women and also their relatives, parents and siblings.

A recent shift in approaches to prevent transmission globally, including in non-endemic countries, recommends the active, systematic screening of girls and women at risk of infection. This will provide excellent opportunities for prevention of transmission throughout pregnancy and birth.

■ PATIENTS AND METHODS

Setting description

Bergamo is a highly industrialized province, with more than one million inhabitants. There is strong agricultural industry throughout the region which includes mountains, lakes, and countryside, Bolivians, from the Cochabamba area, arrived in Italy after 1980; and settled in Bergamo. The two cities have been twinned since 2008 [14, 15].

Currently, the total number of Bolivian population in Bergamo is estimated to be more than 14000, with women making up approximately two thirds. Hospital Papa Giovanni XXIII (HPG) is one of the three public hospitals in Bergamo and is a teaching and referral hospital at national level. Alongside the two public hospitals, there is also a Healthcare Provincial Agency (ATS).

Pregnant women are followed and treated by the outpatient clinics of the hospitals, by the general counseling centers or by private obstetricians.

At HPG, there are also active two active outpatient clinics for Chagas Disease, one for adults and one for babies.

The study was performed between January 2013 and December 2020.

Organization of the project

According to ATS, all the public and private obstetricians were asked to test for Chagas Disease serology in every pregnant woman with Latin American origin. Any pregnant women from Latin America without a serological test for Chagas Disease were investigated during the labor.

All children born to seropositive mothers were

tested after delivery to exclude the presence of *T. cruzi* by standardized micro-methods and were followed within the first month of life. There were further follow-ups at ~6 and ~12 months or until the serological tests were negative.

Newborns were examined to evaluate the clinical manifestations related to symptomatic congenital CD. Skin lesions, neurological signs, respiratory distress syndrome, cardiovascular disorders, and digestive disturbances such as hepatosplenomegaly were analyzed.

The serological test was also proposed to relatives, parents and siblings of infected newborns, and other children of Latin American origin.

Microbiological tests

Serological test

The *T. cruzi* serological test was made using the enzyme-linked immunosorbent assay (Architect Chagas - Abbott Laboratories, Wiesbaden, Germany).

Architect Chagas - Abbott Laboratories is a chemiluminescence immunoassay (CLIA) employing a recombinant multi-antigen protein. The result of the chemiluminescent reaction is an index which is measured in relative light units (RLUs) and expressed as sample RLUs/cut-off value (S/CO). Results were interpreted in accordance with the manufacturer's instructions as follows: positive (S/CO $\geq$ 1.00); grey zone (S/CO between 0.8 and 0.99); and negative result (S/CO $<$ 0.8) [16].

Mothers with positive serological test were considered infected by *T. cruzi*.

Parasitological test

The presence of parasites in mothers and newborns was investigated by thin and thick blood film colored by Giemsa stain directly from blood in EDTA and after concentration by centrifugation or by buffy coat [17].

Polymerase chain reaction (PCR) amplification was performed only once in a suspected infection in a newborn. PCR was performed at Laboratory of Parasitology of University La Sapienza in Rome from a frozen blood sample in EDTA (*T. cruzi* gen-esig[®] Advanced Kit, PrimerDesignTM, UK) [18].

RESULTS

In the study period, 1105 patients were tested by serology for CD: 171 (15%) males and 934 (85%) females, of these 512 were pregnant (Table 1).

The number of serological tests performed was 1388 because some patients underwent more than one test. Some neonates were tested also up to seven times.

As reported in Table 1, 16% (145/934) females and 38% (65/171) males (210/1105 altogether) tested positive at the first serological test.

Of the 60 mothers who tested positive, 28 of the newborns were female and 34 of the newborns were male. The total number of positive newborns was 62 because there were a set of twins. There were a total of 148 positive adults and siblings, which was 14% of all adults and siblings.

Only 2 neonates did not continue the follow up at our laboratory.

As expected, and reported in Figure 1 and Figure 2, all neonates from positive mothers, were positive for serological test for CD at birth.

There were among 144 female and 35 male adults and siblings born between 1991 and 2011, almost all of them tested negative at serology for CD. Only 4 females born between 1991 and 2011, tested positive with a serological test. They were tested at general counseling centers at the ages of 9, 24, 29 and 20 years old respectively.

Three of the women, 24, 29 and 20 years old, were born in Bolivia and were screened as pregnant women. The 9 years old female was born in Italy

Table 1 - Number of patients (females and males) positive or negative for antibodies anti-*Trypanosoma cruzi*.

Antibodies anti- <i>T. cruzi</i>	Females (%)	Males (%)	Adults and siblings (%)	Newborns female (%)	Newborns male (%)	Total patients (%)
	934 (85%)	171 (15%)	1043 (95%)	28 (2%)	34 (3%)	1105 (100%)
Positive	145 (16%)	65 (38%)	148 (14%)	28 (13%)	34 (16%)	210 (19%)
Negative	789 (84%)	106 (62%)	895 (86%)	–	–	895 (81%)

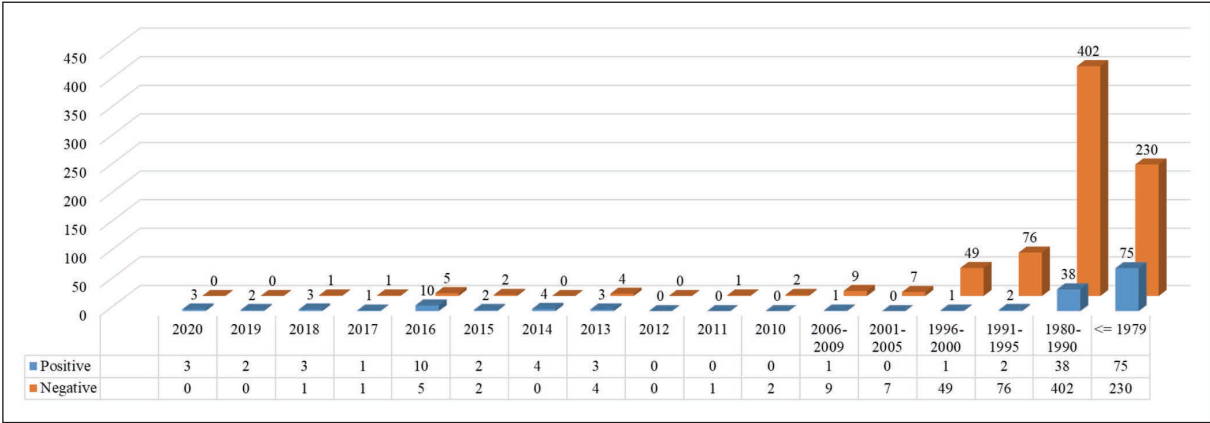


Figure 1 - Number of female patients for date of birth positive or negative for antibodies anti-*Trypanosoma cruzi*.

in 2008 and was screened in 2016 because her mother tested positive to CD serological test while newly pregnant. Even though the 9-year-old female was positive for CD antibodies, she was asymptomatic. She was treated by benznidazole in 2016. For note, the CD prevention protocol was not active in 2008. Among the 62 males born in 1979 or before, 24 (39%) tested positive and 38 (61%) were negative for the serology for CD. Among the 32 males born between 1980 and 1990, 7 (22%) were positive and 25 (78%) tested negative. Among the 305 females born in 1979 or before, 75(25%) tested positive and 230 (75%) were negative for the serology for CD. Among the 440 females born between 1980 and 1990, 38 (9%) were positive and 402 (91%) tested negative.

In Figure 3 and Figure 4, we showed the decrease of S/CO value respectively in female and male neonates. All neonates except one, lost the mother's antibodies before the 12th month and all the lines clearly show a decrease of S/CO value. Only one female neonate showed a result at the CO after 12 months of life. Another test was performed at 450 days, the result was clearly negative. In Figure 3, the follow up of this female neonate is represented by the pale green line that is clearly greater than the other categories after 200 days of life. One neonate, born premature and low birth weight in 2015, as already reported in 2018, was retrospectively diagnosed of *T. cruzi* congenital infection. The direct parasitological tests performed at birth and at 4 weeks tested negative, but he developed recurrent anemia. He was therefore tested for PCR at 32 days of life (tested negative) and

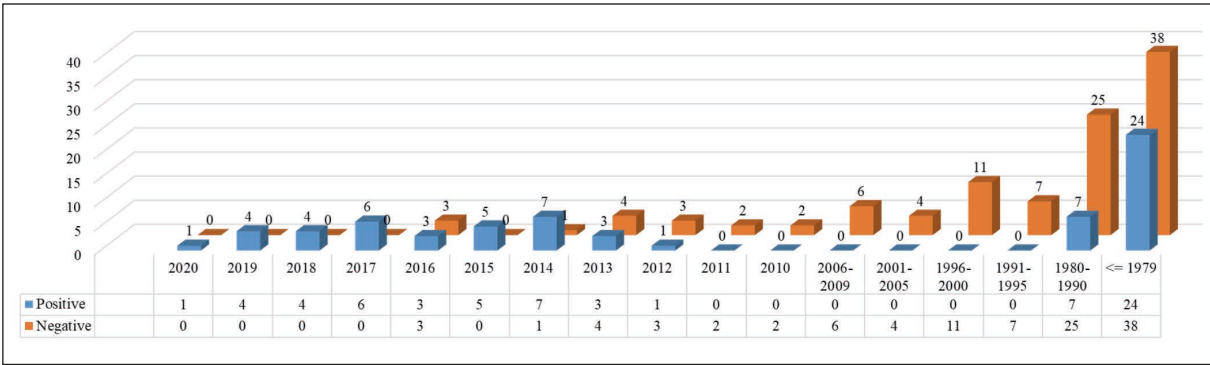


Figure 2 - Number of male patients for date of birth positive or negative for antibodies anti-*Trypanosoma cruzi*.

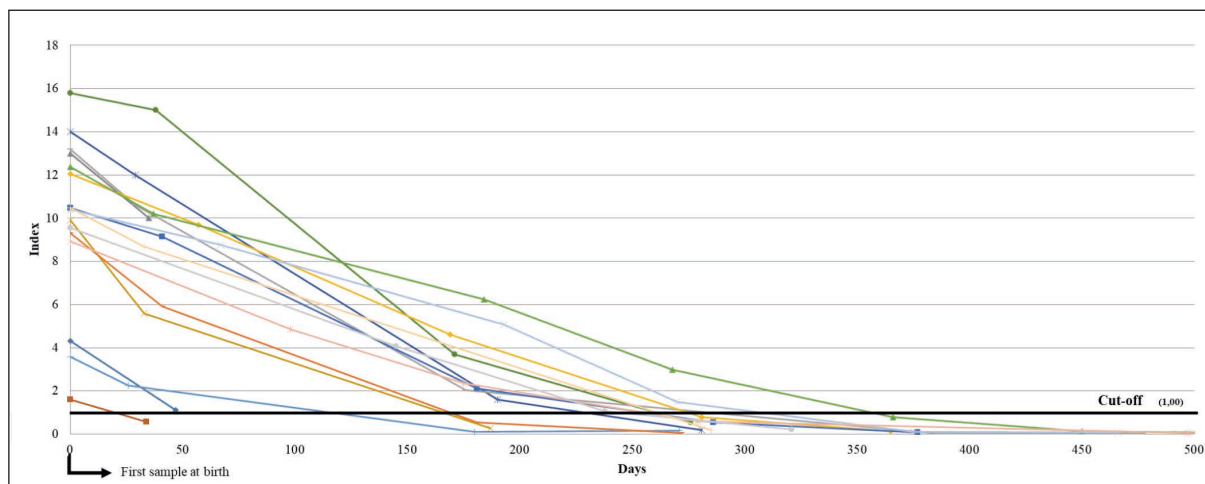


Figure 3 - Follow-up for antibodies anti-*Trypanosoma cruzi* in female neonates.

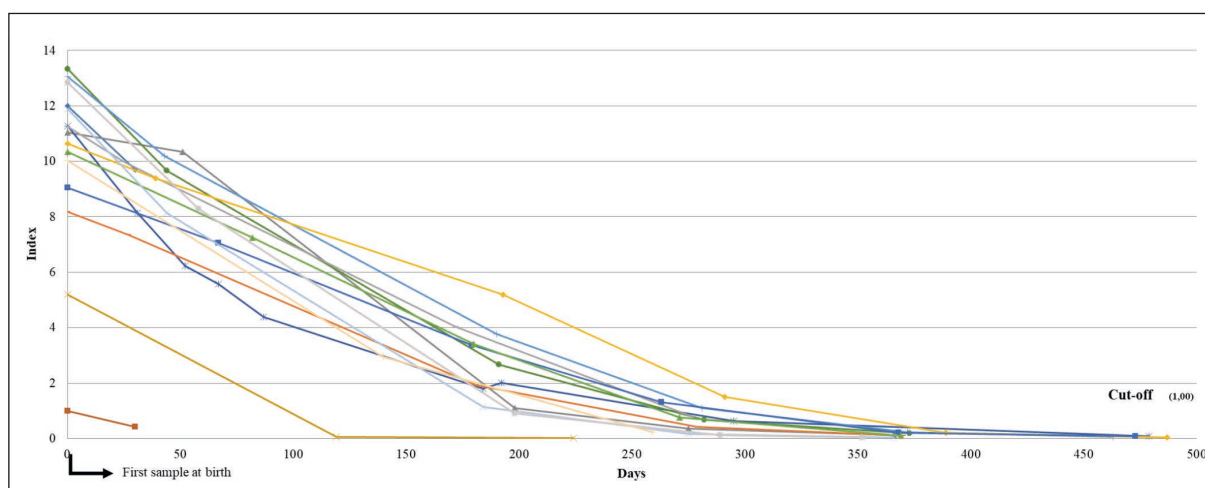


Figure 4 - Follow-up for antibodies anti-*Trypanosoma cruzi* in male neonates.

later at 68 days of life (tested positive). However, the serological tests index values, reported in Figure 5, were decreasing even before treatment administered on June 10, 2015.

DISCUSSION

Due to the migration that has occurred in past decades, Chagas disease has changed its epidemiological profile, becoming more prevalent in urban areas. Although monitoring of blood banks and vector transmission control are essential tools to manage *T. cruzi* infection, prevention and inter-

ruption of congenital CD is a critical step that needs to be solved in order to prevent further disease transmission [19].

Our study was performed following the recommendations of WHO published in 2011 [11].

Our protocol was based on the screening of pregnant women and on the follow up of the neonates. The results enable us to propose some remarks.

1. The positive rate for CD antibodies in adults born before 1979 was 25% for females and 39% for males respectively.

Among those born after 1990 only 4 females tested positive for antibodies against *T. cruzi*.

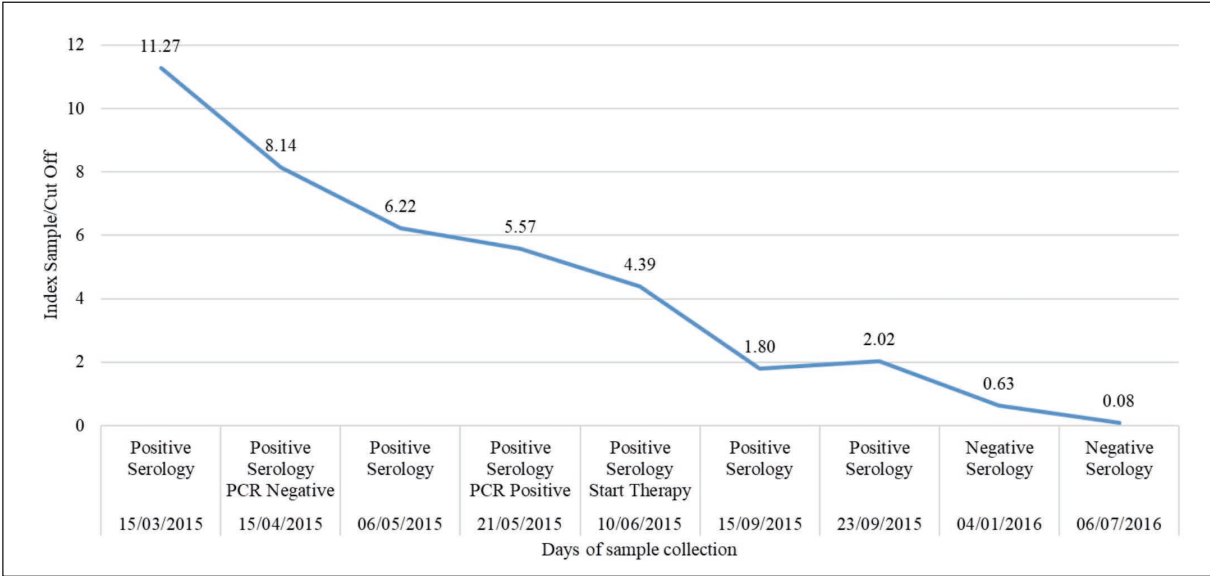


Figure 5 - Serological follow-up of premature baby, with a suspect of infection.

One of them was born in Italy from a mother born in Bolivia in 1981; only 3 women (2%) born in Bolivia after 1990 were positive for CD antibodies. It is interesting that two of the three females were born in 1991 and one in 1999, so only one female born after 1991 was positive for CD antibodies.

There may be some intervention after 1990 which helped to decrease the risk of CD in Cochabamba area.

In order to try to explain the different rate for CD antibodies, we asked to some Bolivians from Cochabamba area, born before 1990, if they could remember any improvement in quality of life, such as changes in houses building; unfortunately, we didn't get a definitive answer.

2. All neonates, with the exception of one, were classified as non-infected according to the follow-up of index value of CD serology.
3. One neonate was diagnosed as infected due to anemia, prematurity, low birth weight and positive PCR in two subsequent tests (even though the 1st PCR tested negative). If we look at the follow-up serology in Figure 5, and we compare it to the corresponding data reported in Figure 3 and Figure 4, we can see that the index value is decreasing in the same way of the other neonates already before the treatment. Only looking at all the follow-up data, it is pos-

- sible to doubt that the premature baby was really a congenital infection. It is currently unclear why the first PCR was negative while the second tested positive. Moreover, the new WHO recommendations underlined that PCR tests are promising and increasingly used (particularly in Europe), though they are not without limitation. False positive results may occur because they lack standardization, leading to various sensitivity levels according to the centers, and quality control programs are still not implemented enough. Therefore, molecular methods require stronger and wider clinical validations before being considered as gold standards to diagnose congenital infection.
4. The CD serology performed by a CLIA test was able to confirm the decrease of index value of antibodies. The index value follow-up could be a good way to try to define if a neonate is not infected. In only one neonate the index value went under 1 S/CO after 360 days. It is necessary in this case to extend the follow-up even longer (more than 360 days) before considering the neonate an infected one.

CONCLUSIONS

This study confirms the usefulness of CLIA tests and the use of the index value as a follow-up met-

ric because the serological test correctly identifies if a neonate is infected or not by observing a decreasing value.

The use of serological follow-up in neonates should be maintained when applying the new WHO recommendation published in 2019.

The difference of positivity rate for CD antibodies between people born before and after 1990 should be investigated further to elucidate useful strategies to further improve the prevention and control of CD.

Conflict of interest

None

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