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Congenital infection with *Trypanosoma cruzi*: from mechanisms of transmission to strategies for diagnosis and control

Conclusions of round tables and synopsis of an International Colloquium

Cochabamba, Bolivia, 6-8 November 2002

Organizers: Yves Carlier (ULB, Brussels, Belgium) and **Faustino Torrico** (UMSS, Cochabamba, Bolivia)

1. Clinical and epidemiological aspects of congenital infection with *Trypanosoma cruzi* (chaired by Gabriel Schmuñis and Faustino Torrico)

1.1. Epidemiological context of congenital infection with Trypanosoma cruzi.

Data were presented on the epidemiological situations of human *Trypanosoma cruzi* infection in the Southern Cone countries of South America (Argentine, Bolivia, Brasil, Chile, Paraguay, Uruguay) and from Peru. In Argentina, Brazil, Chile and Uruguay, there is a decrease in the vectorial transmission by *Triatoma infestans*, the main vector of human infection. In Chile and Uruguay, vectorial transmission is interrupted in all of the original endemic areas, whereas in Argentina it is interrupted in 4 of 18 provinces, and in Brazil in 9 of the 11 states originally endemic for *T. infestans.* Vector control programs of national scope are progressing well in Bolivia and Paraguay, while in Peru, this activity is limited. In all countries, with the exception of Bolivia, the serological covering for *T. cruzi* in blood donors is about 100%.

In Brazil, Chile and Uruguay, because of the above, there is a steady decrease in the prevalence of positive serology for *T. cruzi* in pregnant women (the required but not sufficient condition for congenital transmission of *T. cruzi* infection), when compared with historical data. In the other countries, the prevalence for *T. cruzi* in pregnant women varies from 5 to 40%, depending on the geographical area. Because of migrations, congenital *T. cruzi* infection is increasingly seen in the urban areas and in non-endemic countries. It is a consensus that congenital *T. cruzi* infection is a pressing public health problem at least for the next 30 years, when the pool of infected women of child bearing age will decrease to insignificant levels, at least in the Southern Cone countries. Interruption of vectorial transmission, as well as screening for *T. cruzi* in blood donors, are considered the most effective ways for preventing congenital *T. cruzi* infection.

1.2. Definition of cases of congenital infection with Trypanosoma cruzi.

There is a consensus about the criteria to consider a congenital case: i) the baby has to be born from mother with positive serology for *T. cruzi,* and, ii) parasites are identified at birth, or, iii) parasites or specific antibodies not from maternal origin are detected later after birth, providing that previous blood transfusion and vectorial contamination can be discarded (eg. baby living outside the geographical areas of vectorial transmission).

A presumptive diagnosis can be based on epidemiologic and clinical criteria (see § 1.4), but it can only be confirmed by demonstrating *T. cruzi* and/or the presence of specific antibodies that are not transferred from the mother (see § 2).

1.3. Transmission rates and incidences of congenital infection with Trypanosoma cruzi.

The transmission rate of congenital *T. cruzi* infection (number of congenital cases/number of chagasic mothers) in the Southern Cone countries varies widely, from 1% in Brazil to 4 to 12% Argentina, Bolivia, Chile and Paraguay. These differences were discussed at length, and attributed to the different methodologies used for detection of congenital cases, and/or possible special characteristics from the infecting parasites, differences in the immunological, genetical or nutritional status of the mother, or specific epidemiological situations remaining to be studied.

The need to have, besides the transmission rate, another indicator of the true importance of congenital *T. cruzi* infection in the context of each country, and overall of each epidemiological situation, was discussed. It was suggested that future reports mention the number of congenital cases (numerator) divided by the total number of deliveries that occur in the geographical area in the time period in which the study is made (incidence).

1.4. Clinical aspects of congenital infection with Trypanosoma cruzi.

In most studies (Argentina, Brazil, Chile and Paraguay), 60% to 90% of congenital infection cases were asymptomatic. However, the Bolivian report indicates that symptomatic cases of congenital Chagas disease (having excluded most other possible associated pathologies) were around 50% with a mortality rate of 2 to 14% of infected babies.

The symptomatic cases (so-called *congenital Chagas disease,* whereas *congenital T. cruzi infection*

refers to asymptomatic as well as symptomatic cases) are frequently premature, displaying low birth weight and hepato-splenomegaly. Symptoms of acute respiratory distress (probably related to prematurity) and /or anasarca are seen in some cases. Meningoencephalitis and myocarditis are more frequently observed in cases of co-infection with HIV. There is no specific clinical marker of congenital Chagas disease. In Bolivia, more severe morbidity and higher mortality of congenital Chagas disease are found when mothers live in geographical areas of high vectorial density.

1.5. Recommendations

i) Endemic countries must consider congenital *T. cruzi* infection as a public health problem.

ii) Each endemic country should elaborate an algorithm directed to early detection and specific treatment of detected cases according the capabilities of the local health services and their epidemiological situation (see § 6). Detection and treatment programs for congenital *T. cruzi* infection are currently being implemented in Argentina, Chile, Paraguay and Uruguay.

iii) Vector control programs and serological screening of blood donors have to be implemented since they are the most effective ways for preventing congenital infection.

2. Biological diagnosis of congenital infection with *Trypanosoma cruzi* (chaired by Alejandro Luquetti and Graciela Russomando)

Since the biological diagnosis of congenital infection with *T. cruzi* may theoretically be performed according to various procedures depending on the availability of equipment and expertise of each team, a large discussion was opened about the possible recommendations. A majority of participants agreed with the following:

2.1. Procedures not recommended to ascertain a congenital infection ***at birth*** *(until new progresses are performed)*

i) *T. cruzi*-specific IgG serology, since it does not allow to discriminate antibodies produced by the newborn from those transmitted from the mother;

ii) *T. cruzi*-specific IgM serology, since it is not positive in all congenital cases and is also positive in some uninfected babies born from infected mothers (this might be due to rheumatoid factor and/or materno-fetal transfer of parasitic antigens, cf § 4.3);

iii) *T. cruzi*-specific IgA serology, since it is not positive in all congenital cases.

2.2. Procedures susceptible to be applied to ascertain a congenital infection ***at birth***

The main interest to perform the laboratory diagnosis at birth is to inform rapidly the mother about the positive diagnosis and to explain her the necessity to begin promptly the treatment of her baby (see § 5), without the risk to loose contact with the mother and her baby if they do not come again to the further calls, mainly in countries in which health care is not well organized.

Cord blood seems the best choice for sampling, since it is easy to collect without physical or psychological traumatism for the baby and her mother. If cord blood sampling is not possible, blood can be collected from heel or from peripheral venous puncture of baby.

The direct examination of blood parasites using the microhematocrit concentration method (capillary tubes) can be recommended. The latter is more sensitive than a fresh smear, cheap, affordable and rapid. The sample has to be examined within 24 hours to avoid a decrease of sensitivity due to parasite lysis. The alternative Strout concentration method (so-called microstrout using Eppendorf tubes) can also be used.

Since a positive parasitological result cannot be questioned, alternative indirect parasitological methods, as artificial xenodiagnosis or hemoculture may also be performed if available. However, they are generally more expensive and weeks are necessary before obtaining results, avoiding to give a rapid information to the mother.

PCR can also be a convenient method if a reliable technique is used, but this procedure is generally expensive; It is important to note that all these procedures requires large expertise and skilled personnel.

2.3. Procedures susceptible to be applied to ascertain congenital infection ***after birth***

If the laboratory diagnosis cannot be performed at birth, and whatever the time after birth, it is important to discard possible contamination by other routes as blood transfusion and vectorial contamination, before to ascertain congenital infection in infants (cf 1.2)

Parasitological and some serological methods applied to infant blood can be used in this context:

i) microhematocrit or microstrout concentration methods, other alternative parasitological tests (cf 2.2) or reliable PCR can also be used after birth.

ii) conventional IgG serology allows the diagnosis of congenital infection after the disappearance of maternally transmitted antibodies. Any combination of two techniques can be accepted as ELISA, IF, IHA. There is a consensus that after nine months of age, antibodies passively transmitted from the mother have disappeared; so, a positive serology by this time

indicates *Trypanosoma cruzi* infection of the infant. A practical alternative might be a serology performed at 12 months when vaccines have to be applied to babies. Conventional serology is routinely performed at low costs in most health centers.

iii) detection of IgG antibodies against the recombinant Shed Acute Phase Antigens (SAPA) in babies after three months of age might be an alternative, since maternally transmitted SAPA-specific antibodies would disappear earlier in babies than the conventional antibodies; however, this method has been applied only in Paraguay and reagents are still not available to validate its usefulness.

2.4. Final recommendations

For the laboratory diagnosis of congenital *Trypanosoma cruzi* infection:

i) The serology of the mother should be positive, either by search of conventional antibodies in cord blood, or by performing these examinations in mother before delivery.

ii) the search of parasites in umbilical cord or neonate blood is desirable. If not available, the child should be submitted to a serological analysis at 9-12 months of age. In both cases, positive results will indicate an infection (defined as congenital if blood transfusion and vectorial contamination can be discarded, cf § 1.2).

3. Effect of maternal infection with *Trypanosoma cruzi* on pregnancy outcome (chaired by Faustino Torrico and Yves Carlier)

Belgian data obtained from experimental studies indicated that, depending on the maternal parasitic load, infection with *T. cruzi* in mice can induce severe harmful effects on gestation outcome. However, Bolivian data on asymptomatic mothers chronically infected with *T. cruzi* and having delivered non infected babies indicated that, when there is no materno-foetal transmission of parasites, chronic maternal infection has no effect on human pregnancy and foetal development.

4. Parasite strains, placental pathology and immunological aspects of congenital infection with *Trypanosoma cruzi* (chaired by Yves Carlier and Faustino Torrico)

4.1. Parasite strains and congenital infection with Trypanosoma cruzi

A preliminary study of the belgian-bolivian group, identifying the parasite strains infecting the congenital cases, suggested that, at least in Bolivia, congenital transmission of *Trypanosoma cruzi* does not result from a selection of a particular strain of *T. cruzi*. Studies performed in Chile argues for a more closed relationship between one parasite strain and human congenital infection.

4.2. Placenta and congenital infection with Trypanosoma cruzi

The Belgian team also reported the histo-pathological analysis of placentas from congenital cases of *T. cruzi* infection. If chorionitis and cord oedema were common in placentas from both congenital and control cases, the parasites in placentas of congenital cases were mostly found in chorionic fibroblasts and in subamniotic mesenchyma of the marginal sinus where the membranes attach to the chorionic plate. Contrary to previous observations, no villitis or intervillitis lesions were present, suggesting that the materno-foetal transfer of parasites in these placentas occured by the chorionic route without direct invasion of the trophoblast.

The argentinian group (Cordoba) presented *in vitro* studies on the interactions between *T. cruzi* and the placental alkaline phosphatase (PAP) expressed on syncitiotrophoblast membrane. These results show that alterations of PAP induced with chemical agents reduce the invasion of human placental villi and trophoblastic Hep2 cells by *T. cruzi*, suggesting that PAP might participate to the invasion process of trophoblast when parasites are present in high amounts (10^3 parasites/cells).

4.3. Immunological aspects of materno-foetal relationship in Trypanosoma cruzi *infection*

The belgian-bolivian group reported that cord blood cells of newborns congenitally infected with *T. cruzi* displayed a predominant activation and oligoclonal expansion of CD8 T cells armed to mediate cytotoxic functions. Parasite-specific CD8 T cells secreting IFN-γ were detected. These findings point out that foetuses are more immunologically competent than previously appreciated since, when exposed to alive *T. cruzi,* they are able to develop an adult like immune CD-8 T cell response.

By contrast, uninfected babies born from infected mothers displayed a low level of cord blood T cell activation. However, such neonates presented an overactivation of their innate immune response, since their cord blood cells, and particularly their monocytes, produced higher levels of inflammatory cytokines (IL-1β, IL-6, TNF-α) than controls when stimulated with *T. cruzi* lysate or LPS. They also mounted a strong B cell response, since *T. cruzi*-specific IgM and IgA antibodies were detected in their cord blood. These results show that, even in the absence of congenital infection, maternal *T. cruzi* infection triggers both innate and adaptive immune responses in their foetuses, indicating the materno-

foetal transfer of parasite circulating antigens.

Immunological investigations were also performed in infected mothers showing lower IFN-γ production by parasite-transmitting than non-transmitting mothers when their peripheral blood cells were stimulated with *T. cruzi* parasites. Transmitting-mothers also displayed a higher percentage of positive hemocultures for *T. cruzi* parasites.

From these results, it can be hypothesized that congenital infection (defined by the presence of alive parasites in foetuses and newborns) results from the association of complex phenomenons, such as: i) a weaker maternal Th1 adaptative immune response towards the parasite, ii) a higher parasitic load in maternal blood (see above); iii) a parasite invasion of the placental membranes and umbilical cord (see § 4.2). A CD8 T-cell adaptative response potentially cytotoxic and producing IFN-γ is generated in infected foetuses.

5. Treatment and follow-up of congenital cases of *Trypanosoma cruzi* infection (chaired by João Carlos Pinto Dias and Myriam Lorca)

There was a consensus about the following:

5.1. The specific treatment with the currently available drugs (nifurtimox and benznidazole) is considered obligatory for all cases of congenital *T. cruzi* infection, since it presents a high degree of effectiveness and is very safe for the majority of treated children. Indeed, all infants congenitally infected could be cured with a 100% success if treatment was performed before one year of age, whereas treatments performed after this age, whatever the mechanism of transmission is responsible, have lower possibilities to be successful.

5.2. The fundamental standards for the treatment of congenital *T. cruzi* infection are basically those already established by the World Health Organization in a recent document (WHO. Control of Chagas Disease. WHO Technical Report Series No. 905, Geneva, 2002), which indicates the drug administration as soon as possible for all congenital cases, during 30 to 60 days, in the doses of 5-10mg/kg/day for benznidazole and 10-15mg/kg/day for nifurtimox.

5.3. The treatment can be accomplished at the ambulatory level. However, it is very important to inform the parents and relatives about the correct administration of the drug to assure its best effectiveness and to avoid overdosage and collateral effects. Treated babies must be clinically and biologically followed, ideally each 3 months until 1 year after treatment. Cure assessment is established when conventional serology becomes persistently negative. In the rare congenital cases of therapeutic failure, re-treatment must be considered in each particular circumstance;

5.4. The Round Table emphasizes strongly that the currently recommended drugs must be available in all the endemic countries. The Assembly reports a great variety in the prices of these drugs in the different countries of the Region, not justified by the costs of elaboration in these countries. It strongly recommends that the current prices should be reviewed by PAHO, country authorities and the producers, looking for a regional policy of equity. Moreover, a liquid (paediatric) presentation of the drugs should be formulated by the producers to make easier the treatment of such congenital infections.

5.5. Strong recommendations of the Assembly (unanimous approval)

The Assembly considers the treatment of congenitally infected newborns and children as fundamental. It asks the availability of specific drugs at uniform costs in all the concerned countries of Latin America, ideally in a paediatric formulation.

6. Recommendations for a control strategy of congenital *Trypanosoma cruzi* infection (chaired by João Carlos Pinto Dias and Gabriel Schmuñis)

6.1. The Round Table recognises that congenital *T. cruzi* infection occurs in all the endemic areas, with different intensities and characteristics in each sub-region, as well in non-endemic areas (see § 1). It requires particular and institutional attention from each government, in terms of Public Health Policy and investigation. Research needs and practical questions concerning congenital *T. cruzi* infection must be focused with priority by research teams and should be discussed in seminars or workshops such as the present one.

6.2. The Assembly reminds that specific treatment of the mother during pregnancy should not be performed (including in acute cases) with the currently available drugs, since their teratogenic risk is not known and their efficiency in chronically infected individuals is low (see § 6.1). Research on new drugs for the specific treatment of *T. cruzi* (chronic) infection and safe during gestation is therefore strongly recommended.

6.3. Since no specific direct prevention of congenital *T. cruzi* infection is available (see above), the best strategies concerning this problem are divided in two categories: i) the systematic and effective control of the basic primary transmission routes of the infection in endemic areas (mainly the vectorial and transfusional routes), in order to reduce the prevalence rate of infected women (see § 1), and, ii) the early and correct diagnosis of congenital cases (see § 2), in order to treat infected newborns/neonates, so avoiding congenital Chagas disease-related mortality and morbidity in the neonatal period or later in life.

6.4. Special attention must be paid to the diagnosis of congenital *T. cruzi* infection (see § 2) and its correlated public health implications, considering the following possibilities/strategies:

i) The ideal schedule is to institutionalize conventional serology for pregnant women in endemic areas and to follow the infected ones and their babies, with the main objectives of adequate mother care and early detection and treatment of congenital infection;

ii) For babies delivered from mothers serologically positive or with known antecedents of *T. cruzi* infection, or for all babies born in endemic areas where pre-natal mother examination is impossible, the suggestion is to perform either direct parasitological examination of the umbilical cord blood (microhaematocrit, microstrout), or indirect parasitological examination by hemoculture and/or artificial xenodiagnosis, or molecular examination of cord blood by reliable PCR techniques, or a first IgG conventional serology allowing further serological follow-up (cf. § 2). In the case of negative direct parasitological tests in babies with strong clinical suspicion of congenital Chagas disease, it is suggested to repeat these tests;

iii) In terms of Public Health programmes, another possibility would be the simple conventional serology of children in the age of 9-12 months, considering infected and candidate for treatment all those cases with positive results (cf § 2);

iv)Since the early diagnosis of congenital *T. cruzi* infection is highly desirable, new diagnosis techniques and strategies must be investigated, such as more reliable and simple procedure of PCR and the utilization of recombinant technology for antigen or antibody detection.

6.5. The babies with positive results derived from either direct or indirect parasitological examinations, reliable PCR or serology at 9-12 months of age must be treated with specific drugs, as indicated above (cf 5). Negative babies for parasitological tests must be clinically followed until the age of 9 months, when a new conventional serology must be performed. Negative cases at this time are considered non infected. Positive serology, on the other hand, will indicate active infection and the child will be treated.

6.6. Reinfection of treated and cured babies is possible and has been registered in endemic regions where housing infestation by triatomines occurs. So, an institutional program against congenital *T. cruzi* infection in endemic areas must always be associated with regular activities of vector and blood banks control (cf § 6.3).

6.7. Each detected case of congenital *T. cruzi* infection presupposes an epidemiological investigation in the whole family in terms of Public Health Policy, in order to detect other infected children and to make possible an epidemiological evaluation of the situation, also providing medical attention to other eventually infected relatives.

6.8. In a particular way, infected mothers deserve special attention concerning future possible pregnancies because of the risk of repeated foetal infections in children of subsequent pregnancies. Moreover, it is remembered that the possibility of heart Chagas disease must be investigated in order to prevent complications during pregnancy and birth.

The conclusions of the round tables and synopsis of this colloquium were obtained through the participation of the following national representatives:

Argentine: Beatriz Basso, Edgardo Moretti, Pedro Moya and Maria José Sartori (Universidad Nacional de Cordoba), Héctor Freilij (Hospital de Niños “Ricardo Gutierrez”, Buenos Aires), Sergio Sosa-Estani (Ministerio de Salud, Buenos Aires).

Belgium: Yves Carlier, Sergio Fernandez Aguilar, Emmanuel Hermann, Carine Truyens and Michal Svoboda (Université Libre de Bruxelles).

Bolivia: Cristina Alonso Vega, Claire Billot, Patricia Rodriguez, Eduardo Suarez, Faustino Torrico, Mary-Cruz Torrico and Myrna Virreira (Universidad Mayor de San Simon, Cochabamba), Abraham Jemio, Miguel Delgado and Miguel Torres (Ministerio de Salud y Prevision Social**,** La Paz).

Brazil: Alejandro Luquetti (Universidade Federal de Goias, Goiânia), João Carlos Pinto Dias (Universidade Federal de Minas Gerais, Belo Horizonte), Aluizio Prata (Faculdade de Medicina do Triangulo Mineiro, Uberaba), Honour President.

Chile: Maria Inés Bahamonde and Myriam Lorca (Universidad de Chile, Santiago).

Paraguay: Graciela Russomando (Instituto de Investigaciones en Ciencias de la Salud, Asunción).

Peru: Cesar Naquira (Instituto Nacional de Salud, Lima).

USA: Gabriel Schmuñis (PAHO/OPS, Washington).

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