

MINIMUM CHANGE IN HEMOCULTURE PROTOCOL SIGNIFICANTLY IMPROVES ITS POSITIVITY AND CONCORDANCE WITH SEROLOGICAL AND MOLECULAR RESULTS IN CHRONIC CHAGAS DISEASE

ANGÉLICA SAYURI MIZUTANI¹, ANA FLÁVIA DE ARRUDA PIOVESANI², MARTA BÉRTOLI³, ÉRIKA CRISTINA FERREIRA⁴, MÁRCIA MACHADO DE OLIVEIRA DALALIO⁵, DIVINA SEILA DE OLIVEIRA MARQUES⁶, MAX JEAN DE ORNELAS TOLEDO⁷, SILVANA MARQUES DE ARAÚJO⁸, MÔNICA LÚCIA GOMES^{9*}

1. Master's Degree in Biosciences Applied to Pharmacy, State University of Maringá, Paraná, Brazil; 2. Master's Degree in Science in Health, State University of Maringá, Paraná, Brazil; 3. Master's Degree in Clinical Analyzes, State University of Maringá, Paraná, Brazil; 4. Professor, Master, Department of Statistics, State University of Maringá, Paraná, Brazil; 5. Professor, Ph.D, Department of Basic Health Sciences, State University of Maringá, Paraná, Brazil; 6. Professor, Ph.D, Regional University Hospital of the North of Paraná, State University of Londrina, Paraná, Brazil; 7. Professor, Ph.D, Department of Basic Health Sciences, State University of Maringá, Paraná, Brazil; 8. Professor, Ph.D, Department of Basic Health Sciences, State University of Maringá, Paraná, Brazil; 9. Professor, Ph.D, Department of Basic Health Sciences, State University of Maringá, Paraná, Brazil.

* Department of Basic Health Sciences - State University of Maringá. Av. Colombo, 5790. Maringá, Paraná, Brazil. ZIP CODE: 87020-900. mlgomes@uem.br

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ABSTRACT

Extending the 120-day cultivation period to 180 days increased the positivity of hemoculture 11 times (1,100% increase; $p = 0.0052$) in 87 patients with chronic Chagas disease. This change improved the concordance with the enzyme-linked immunosorbent assay and polymerase chain reaction results and may be an important strategy in cases of very low parasitemia.

KEYWORDS: *Trypanosoma cruzi*, hemoculture, positivity increase, PCR, ELISA.

1. INTRODUCTION

Latin America has 5-6 million cases of Chagas' disease¹. Through the migration of these individuals to non-endemic countries, this disease has become a global health problem².

Hemoculture is one method that is available to detect the parasite, but it has low sensitivity^{3,4} that depends on the number of samples collected, amount of blood sampled, time between collection and blood processing, cultivation time, reagents used, and experience of the professional^{3,5-9}. Three successive samplings per patient and elongation of the cultivation time were shown to increase the positivity of this method⁵⁻¹⁰. Polymerase chain reaction (PCR) is another method that is used to detect the parasite. Although more sensitive than conventional parasitological methods¹¹⁻¹³, PCR is not recommended in routine laboratory practice because of possible disagreements with serological results, the need for a differentiated infrastructure and specialized training, and high

cost^{14,15}. Improving the positivity of existing conventional methods is important. In the present study, the cultivation time for hemoculture was increased to 180 days to raise its positivity and improve correlations with serological and molecular findings in patients with Chagas' disease.

2. MATERIAL AND METHODS

The study involved 87 patients with positive enzyme-linked immunosorbent assay (ELISA) findings from the Chagas' Disease Laboratory of the State University of Maringá (UEM) and Clinical Hospital of the University of Londrina, Paraná, Brazil. The majority (57.5%) of the patients were female. The average age was 58.7 ± 9.8 years in patients with negative hemoculture and 63.7 ± 11.4 years in patients with positive hemoculture. The patients signed a free and informed consent form that was approved by the Permanent Committee of Ethics in Research Involving Human Beings (COPEP) of UEM (protocol no. 012/2010).

The hemocultures were processed according to Chiari et al.⁶, but the cultivation time was extended from 120 to 180 days. Parasitemia in the patients was classified according to the number of positive tubes in accordance with other studies^{7,8} (5-6 positive tubes [high], 3-4 positive tubes [medium], 1-2 positive tubes [low], 0 positive tubes [null]) and time required for detection of the parasite (90 days [high], 150 days [medium], and 180 days [low]). Polymerase chain reaction (PCR) was performed as previously described^{11,16}. The data were analyzed us-

ing Statistica 8.0 software. The *t*-test was used to compare the average age of the patients with positive and negative hemocultures, and the χ^2 test was used to verify the increase in the positivity of the hemoculture and associations between the methods. The level of statistical significance was 5% ($p < 0.05$).

Ethical Approval: This study was approved by Permanent Committee of Ethics in Research Involving Human Beings (COPEP) of UEM, under protocol number 012/2010.

Conflicts of interest: The authors declare that they have no conflicts of interest.

3. RESULTS

The positivity of the hemoculture was 1/87 (1.15%) up to 120 days of observation and 11/87 (12.65%) up to 180 days, representing an increase of 1,100% ($p = 0.0052$). By increasing the cultivation time by 33%, the positivity of the hemoculture increased 11 times. The highest positivity (11/12 [91.7%]) occurred at 150 and 180 days. Most patients (7/12 [58.3%]) had positive hemoculture results at 180 days (low parasitemia), followed by 4/12 (33.3%) with positive results at 150 days (medium parasitemia), and 1/12 (8.3%) with positive results at 90 days (high parasitemia).

Table 1 shows parasitemia according to the number of positive tubes. Of the patients with positive hemoculture (12/87), 75.0% (9/12) had low parasitemia, 16.7% (2/12) had medium parasitemia, and 8.3% (1/12) had high parasitemia. The comparison of the results that were obtained from the two parameters that were used to classify parasitemia showed 58.3% agreement (7/12). No parasitemia was detected in 75 patients (86.2%).

Table 1. Parasitemia in 87 individuals infected with *Trypanosoma cruzi* according to the number of positive hemoculture tubes.

Patients n (%)	Positive tubes	Parasitemia
75 (86.2)	0/6	Null
8 (9.2)	1/6	Low
1 (1.1)	2/6	Low
2 (2.3)	3/6	Medium
1 (1.1)	5/6	High
87 (100)		

Table 2. Association between hemoculture and PCR results in 87 patients infected with *Trypanosoma cruzi*.

Infected with <i>Trypanosoma cruzi</i> .					
Method	Hemoculture with 180-day cultivation				<i>p</i>
	Negative (<i>n</i> = 75)		Positive (<i>n</i> = 12)		
	<i>n</i>	%	<i>n</i>	%	
PCR					
Negative	53	60.9	3	3.4	0.00216*
Positive	22	25.3	9	10.3	

*Association at 5% level of significance (χ^2).

Seventy-five percent of the patients (9/12) with posi-

tive hemoculture had positive PCR results, and 70.7% patients (53/75) with negative hemoculture had negative PCR results, indicating a significant association ($p = 0.00216$) between these two methods (Table 2). The positive hemocultures showed 100% agreement with the ELISA results.

4. DISCUSSION

In the present study, the cultivation time for hemocultures of single blood samples was extended to 180 days, which significantly increased positivity. In a previous analysis of cultivation times up to 120 days⁶, only one patient (1.15%) showed positive results, with detection of the parasite at 90 days of cultivation. In another study, an increase in positivity was observed with one or three hemocultures, with a lower blood processing time, mild homogenization, and analysis up to 120 days¹⁷. Other authors also reported a higher number of positive hemocultures with evaluations of 120-150 days⁷⁻⁹. The number of positive hemocultures in the present study increased as the cultivation time increased: 400% at 150 days (four patients) and 700% up to 180 days (seven patients). Improvements in the positivity of hemocultures have been proposed since the 1950s^{5,7,8}. Different protocols⁵ relative to the most widely used protocol in the literature⁶ also demonstrated increases in the number of positive hemocultures up to 180 days.

Successive samplings per patient have been shown to increase the sensitivity of hemocultures^{7,8,17,18}, but performing such repetitive samplings is laborious for both the patient and laboratory, in addition to increased costs. In the present study, we used a technique that involved a single blood sample with an increase in the cultivation time. The results showed that a significantly higher percentage of the hemocultures were positive. This methodological modification increased positivity without the need to perform the protocol several times, thus reducing the number of patient visits to the laboratory and easing the costs associated with multiple testing. The limitations of the present technique include an increase in hemoculture storage time, a prolonged time of detection and isolation of the parasite, a greater number of analyses that to be performed, and more manipulations of the material that may involve a risk of contamination.

The positivity of hemocultures at 150-180 days of cultivation appeared to be related to the level of parasitemia, in which most patients had low or medium parasitemia, rather than related to the intrinsic biological characteristics of the parasite. The parasites that were isolated from these patients belong to distinct typing unit (DTU) TcII¹⁹, which has a high multiplicative ability and low replication time^{20,21}, indicating that the higher cultivation time that is required for detection is likely related to the number of parasites relative to the onset of incubation, which is consistent with low parasitemia and the

high percentage of negative hemocultures.

A significant association was found between the hemoculture and PCR results in both the positive group and negative group. Nonetheless, discordant results were observed. Patients with positive hemocultures had negative PCR results, likely because of the presence of inhibitors in the sample and intermittent parasitemia in the bloodstream^{8,22,23}. Patients with negative hemocultures had positive PCR results, demonstrating greater sensitivity of PCR as previously reported^{11-13,24-26}.

5. CONCLUSION

In conclusion, extending the hemoculture cultivation time to 180 days increased its positivity. It also increased the concordance with the ELISA and PCR results, which may indicate an important diagnostic strategy for patients with very low parasitemia.

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