Using VFDB, Kleborate and BLAST tools, the genome was screened for virulence genes. The genes yersiniabactin *(ybt)*, colibactin *(clb)*, salmochelin *(iro)*, regulator of mucoid phenotype A *(rmpA)* and aerobactin *(iuc)* were detected as virulence factors. The detection of the OXA-48-like carbapenemase in the resistome using rgi-CARD,[5] without associated extended-spectrum beta-lactamase, was important. Using the PlasmidFinder database,[6] the plasmids IncL, IncFIB(K) and IncFIA(HI1) were found. Plasmids IncL and IncFIB(K), along with other types of replicon, have been identified as carriers of carbapenem-resistance genes[7] and the IncL/M is specifically involved in the worldwide spread of bla_{OXA-48}[8]. Finally, the sequences were deposited in BIGSdb-Pasteur, which assigned a new sequence type: ST6423, and are available in NCBI GenBank® (GCA_037150565.1, BioProject ID: PRJNA1078582).

The presence of HvKp with its distinctive hypermucoviscosity, especially in the context of biomedical device-related infections, poses a challenge due to its ability to form biofilm. This phenomenon, associated with OXA-48-like carbapenemase, complicated the management and treatment of the patient.

The detection of HvKp cases justifies exhaustive genomic surveillance, as its characterisation is crucial to understanding both its genetic drift and the resistance mechanisms it may acquire. Ultimately, the comprehensive study of HvKp provides an opportunity to address the clinical and epidemiological challenges associated with these microorganisms.

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HIV and Chagas disease coinfection

Enfermedad de Chagas y coinfección por el VIH

American trypanosomiasis or Chagas disease is caused by *Trypanosoma cruzi (T. cruzi)*, a flagellated protozoan which is transmitted through the inoculation of faecal matter from triatomines. It can also be transmitted through blood products, organ transplants, orally or by transplacental transmission. If the acute form is not treated, the parasite remains latent and can lead to visceral complications in 30%–40% of people throughout their lives. In the case of immunosuppressed individuals, particularly in cellular immunosuppression, there is a risk of reactivation, which can cause serious complications with high mortality rates.[1,2]

We describe a case of *T. cruzi*-HIV coinfection in a patient with severe immunosuppression, suboptimal treatment with benznidazole and temporary discontinuation of antiretroviral treatment, with subsequent positive PCR for *T. cruzi*. The patient was a male in his sixties from Bolivia, diagnosed in September 2009 with stage C3 HIV infection ($CD4^+$ 59 cells/mm^3, 1,6%) manifesting with *Pneumocystis jirovecii* pneumonia and *Cystoisospora belli* diarrhoea, which were successfully treated for three weeks. During his admission he was also diagnosed with chronic Chagas disease in an indeterminate phase (positive ELISA and haemagglutination serology; positive *T. cruzi* PCR). Routine cardiology tests (electrocardiogram and echocardiogram) at initial screening were normal.

He was started on antiretroviral treatment (ART) with efavirenz/emtricitabine/tenofovir disoproxil (Atripla®), with progressive immune recovery, reaching $CD4^+$ lymphocyte counts greater than 200 cells/mm^3 after one month of treatment, achieving a $CD4^+$ lymphocyte percentage of around 10% (Fig. 1). In November 2010, after one year of ART and a CD4 $count^+$ of 161 cells/mm^3 (9.5%), it was decided to start treatment for Chagas disease with benznidazole at a dose of 5 mg/kg/day. The treatment had to be discontinued after 15 days due to skin toxicity. PCR for *T. cruzi* was negative two months after stopping the treatment. During follow-up, an annual electrocardiogram, six-monthly echocardiogram and a cardiac magnetic resonance imaging scan were requested with no evidence of visceral involvement. The patient also did not develop any gastrointestinal symptoms during this time. After a progressive immune recovery $CD4^+$ 403 cells/mm^3 (20%), in November 2022, the patient stopped ART for two months, experiencing a viral rebound of up to 4.39 log and a decrease in $CD4^+$ lymphocytes of 282 cells/mm^3 (7.8%). At that time, a new

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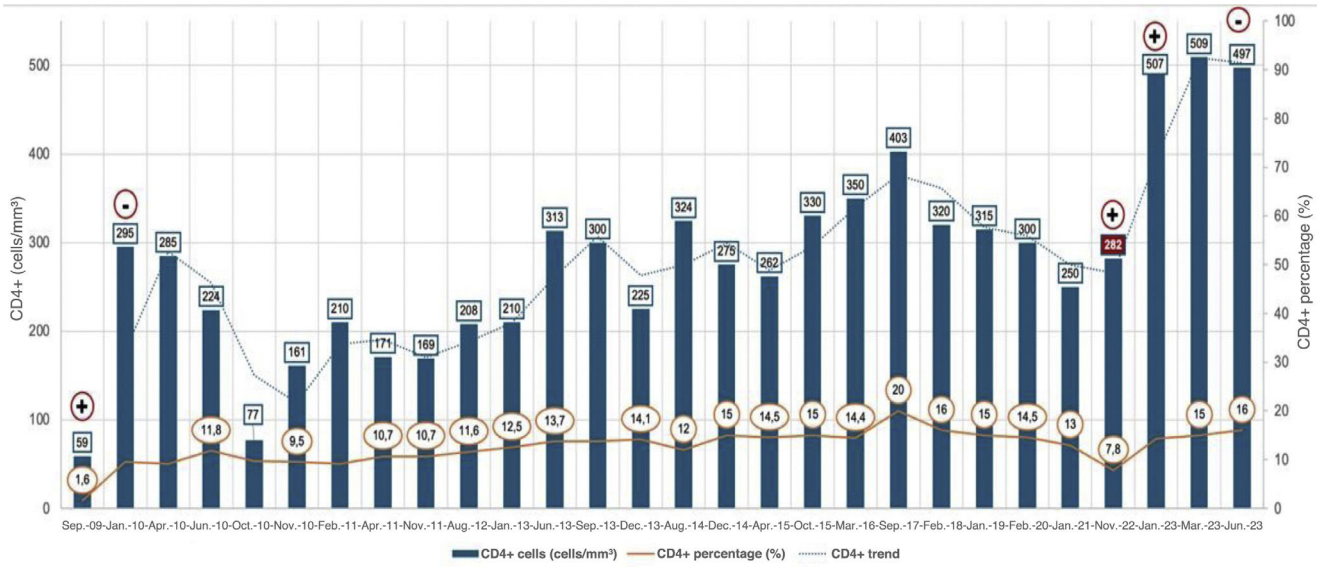

Fig. 1. The patient's immune status over time. Note the positive PCR *(O)* for *T. cruzi* at diagnosis and after immunological deterioration.

PCR determination for *T. cruzi* was performed, which was positive. The ART was resumed with the regimen he was taking at that time, dolutegravir/lamivudine (Dovato®), achieving viral suppression once again and CD4$^+$ immune restoration to 497 cells/mm^3 (16%) up to his last check-up in September 2023. Despite this improvement in the patient's immune and virological status, two other positive serial (from September to January 2024) PCR determinations for *T. cruzi* were found. Microbiological examination was extended with a blood study using Giemsa stain and Strout concentration test, with negative results. At no time did he show symptoms suggesting reactivation. At this time, a new treatment for Chagas disease was proposed with nifurtimox (8 mg/kg/day) and this was completed, although with several interruptions in the second month which extended the course of treatment by 15 days. The follow-up PCR after treatment was negative and remains so to date. His immune and virological status remained stable with undetectable viral load and CD4$^+$ lymphocyte counts of 416 cells/mm^3 (20%).

Chagas disease is endemic in continental Latin American countries. However, as a result of migration it has spread throughout the world, especially to Europe, where Spain is the country with the highest prevalence; in endemic areas the rate of *T. cruzi*-HIV coinfection is estimated to be between 1.3% and 7.12%.[3] Reactivation of chronic Chagas disease occurs mainly in people with cellular immunosuppression and manifests as meningoencephalitis or myocarditis, with high morbidity and mortality rates.[4,5] In patients with Chagas disease co-infected with HIV, reactivation usually occurs when the CD4 count$^+$ drops to less than 200 cells/mm^3. The risk of reactivation without ART is 15%–35%.[4,6] However, a positive blood PCR result is not considered a definitive criterion for reactivation, as in 30%–70% of patients under follow-up this commonly occurs during the indeterminate chronic phase.[4,5] For the diagnosis of reactivation in people with HIV, direct visualisation of trypomastigotes in peripheral blood or CSF, detection of positive PCR for *T. cruzi* in CSF or histological findings with the presence of inflammatory foci and amastigotes are necessary, usually being accompanied by clinical manifestations such as fever, meningoencephalitis, myocarditis or skin lesions.[3,5]

To reduce morbidity and mortality rates, it is recommended to start antiparasitic treatment early, with benznidazole 5 mg/kg/day being the treatment of choice or, alternatively, nifurtimox 8–10 mg/kg/day for 60 days. In cases of severe meningoencephalitis, benznidazole doses may be increased up to 10 mg/kg/day. No cases of immune reconstitution inflammatory syndrome have been reported with early initiation of ART, so there is no need to wait until the end of antiparasitic treatment to administer ART.[3,7] The main problem with benznidazole treatment is the high rates of adverse effects (up to 52%) in adults, and treatment interruptions (up to 14%).[8] Clinical trials have been conducted with shorter regimens, of up to two weeks, with the aim of reducing toxicity and facilitating treatment.[9,10] The main limitation of these studies is that sustained negativity of *T. cruzi* PCR is used as the outcome. Although this strategy allows for clinical trials with manageable samples and follow-up periods, PCR techniques have not been shown to be surrogate markers of cure, at least not in moderate/advanced Chagas disease.[11]

Chagas disease should be screened for in immunosuppressed individuals from endemic areas due to the risk of disease progression and the possibility of reactivation. If detected, treatment is highly recommended, preferably with benznidazole. The duration of treatment in chronic Chagas disease is currently subject to debate. However, until more information is available on the clinical efficacy of treatment regimens shorter than two months, it is advisable to err on the side of caution, especially in patients with immunosuppression or at risk of becoming immunosuppressed.

CRediT authorship contribution statement

All the authors made substantial contributions to each of the following: 1) data collection; 2) critical review of the intellectual content; and 3) final approval of the version submitted.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Analysis of the serological diagnosis of syphilis: A proposal for improvement

Análisis del diagnóstico serológico de la sífilis: una propuesta de mejora

Serological techniques are the gold standard for the diagnosis of syphilis, with the reverse algorithm being the most widely used. This consists of an automated treponemal screening test, followed by a second confirmatory treponemal test and a third non-treponemal test. However, since it was published in the early 2000s, this algorithm has barely been modified.[1,2] Numerous studies now point to the utility of the index provided by screening platforms for predicting infected patients who do not require additional confirmatory tests, and for predicting potential false positives.[3] However, for application in routine clinical practice, this index has to be validated for each individual diagnostic screening platform.

In view of the above, we set out to determine the relationship between the index value, expressed as a signal-to-*cut off* (S/CO) ratio, of samples reactive for the Alinity Syphilis TP assay (Abbott Diagnostics, Abbott Park, IL) based on chemiluminescent microparticle enzyme immunoassay (CMIA), and the result of a second confirmatory treponemal test. To our knowledge, this is the first evaluation of these characteristics performed with the Alinity Syphilis TP assay.

We studied serum samples positive for the Syphilis TP assay processed in two third-level hospitals, corresponding to the two health areas of Valladolid, over a two-year period (January 2022–January 2024). A sample with an index value ≥1 S/CO was considered positive. Depending on the processing centre, confirmatory treponemal techniques available during the study period were INNO-LIA Syphilis Score (Fujirebio, Gent, Belgium) or TPHA (Master Labor SL, Madrid, Spain). We consider a true positive (TP) to be a patient with a positive Syphilis TP and positive confirmatory test, and a false positive (FP) to be a patient with a positive Syphilis TP and a negative or indeterminate confirmatory test, confirmed with a second serum sample. We excluded patients with positive Syphilis TP and negative confirmatory test, in whom a second serum sample was not analysed. Continuous quantitative variables were expressed as median and interquartile range (IQR). The relationship between the S/CO of the Syphilis TP assay and the outcome of confirmatory screening tests was assessed using ROC curves. Statistical differences were evaluated using the Mann–Whitney U test ($p < 0.05$).

We included 1676 Syphilis TP assay-positive samples, corresponding to 832 patients. The median age was 43 (IQR: 33–56) and 76% were male. The confirmatory techniques used were INNO-LIA-Syphilis Score in 42.9% of cases and TPHA in 57.1%. A total of 776 patients (93.3%) were diagnosed as true positives and 56 (6.7%) as false positives. The median for true positives was 17 S/CO (IQR: 11–20.6) while for the false positives it was 2.4 S/CO (IQR: 1.6–2.9) ($p < 0.001$). ROC analysis revealed that 100% of patients with a screening test score greater than 10.37 S/CO were true positives, so it would not have been necessary to perform confirmatory tests and, taking into account the price of these techniques (20.57 euros for the INNO-LIA Syphilis Score and 2.05 euros for the TPHA), this would have meant a saving of 11,993.70 euros in our study period (Fig. 1). In terms of reducing the percentage of false positives, the ROC analysis showed an optimal cut-off point of 5.88 S/CO

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