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Postoperative Serological Monitoring after Conservative Surgery for Hepatic Cystic Echinococcosis: A Prospective Cohort Study

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Abstract

Background

Postoperative serologic follow-up after surgery for hepatic cystic echinococcosis (HCE) is widely used, but the kinetics of antibody decline and the factors influencing time to seronegativation remain poorly characterized. This study aimed to describe the postoperative evolution of hydatid serology, characterize its kinetics during long-term follow-up, and evaluate its practical contribution to post-therapeutic surveillance.

Methods

We conducted a prospective, single-center cohort study of 60 patients (99 hepatic hydatid cysts) who underwent standardized conservative surgery for HCE between June 2017 and December 2023, with a median follow-up of 61 months. Enzyme-linked immunosorbent assay (ELISA) and indirect hemagglutination assay (IHA) were performed preoperatively and at scheduled postoperative intervals. Time to seronegativation was analyzed using Kaplan-Meier estimates and Cox regression; longitudinal titer kinetics were analyzed using segmented regression for repeated measures.

Results

Among initially seropositive patients, seronegativation occurred late, with a median of 65 months for ELISA and 71 months for IHA, and more than 60% of patients remained seropositive at 5 years. Serologic titers followed a biphasic pattern, with an early transient rebound (3.04 months for IHA, 6.67 months for ELISA) followed by a significant linear decline (− 2.4 dilutions/month for IHA and − 0.01012/month for ELISA; $P < .001$ for both). On multivariate Cox regression, preoperative C-reactive protein and initial dual ELISA/IHA positivity were the only factors independently associated with time to seronegativation.

Conclusions

Postoperative serologic decline after HCE surgery is slow, and isolated persistent seropositivity should not, by itself, be interpreted as recurrence. A persistent secondary re-elevation of titers after the postoperative rebound should raise suspicion of recurrence and prompt radiological re-evaluation.

Background

Cystic echinococcosis (CE) is a parasitic zoonosis that represents a major public health problem worldwide and particularly in Tunisia (1). The global prevalence is estimated at more than one million infected individuals, with approximately 200,000 new human cases occurring each year, and an incidence ranging from 1 to 50 cases per 100,000 inhabitants depending on the region (2). In Tunisia, where the disease remains endemic, the surgical incidence reaches 12.7 cases per 100,000 inhabitants per year (3). In humans, any organ may be affected; however, the liver remains the most frequent site, accounting for

approximately 50% to 70% of cases (4). According to the World Health Organization (WHO) guidelines (2025), the diagnosis of cystic echinococcosis relies primarily on imaging, particularly ultrasonography, with serology playing only a complementary role and being mainly indicated when imaging findings are inconclusive (2).

Postoperatively, follow-up relies primarily on clinical and, above all, radiological assessment. Serology may contribute to surveillance, but its value is limited by antibody persistence and by variable performance depending on disease stage, cyst location, and treatment history. Isolated seropositivity is therefore insufficient to conclude recurrence, underscoring the value of analyzing serologic kinetics and correlating them with imaging findings (4).

In this context, the objective of our study was to describe the evolution of hydatid serology after surgery for hepatic cystic echinococcosis (HCE), to characterize its kinetics during follow-up, and to assess its practical contribution to post-therapeutic surveillance.

Methods

Ethics Committee Approval and Patient Consent

The study protocol was approved by the local ethics committee of Habib Bourguiba University Hospital, Sfax (CPP Sud No. 0021/2017), and all patients provided written informed consent before inclusion. The study was conducted in accordance with the principles of the Declaration of Helsinki. The trial was registered in the Pan African Clinical Trials Registry (PACTR) under the registration number PACTR202608878713534 on August 3, 2026.

Study Design

We conducted a prospective, descriptive, and analytical, single-center study carried out from June 1, 2017, to December 31, 2023, in the Department of Visceral and Digestive Surgery at Habib Bourguiba University Hospital (Sfax, Tunisia). The study population comprised 60 patients operated on for HCE using a standardized conservative technique.

All patients underwent a standardized conservative surgical procedure. According to the approved protocol, this included liver mobilization for dome-located cysts, intraoperative ultrasound assessment, cyst evacuation, intraoperative methylene blue testing to detect cystobiliary fistula, and omentoplasty whenever technically feasible.

Patients who underwent elective conservative surgery for HCE according to a standardized protocol were included. Patients operated on emergently, those who underwent radical treatment (total or subtotal pericystectomy or hepatectomy), cases operated on outside the standardized protocol, and patients lost to follow-up whose data could not be exploited were not included.

Perioperative Parasiticidal Treatment

No patient received preoperative albendazole. Postoperatively, all patients received albendazole at a dose of 15 mg/kg/day, divided into two daily doses, for a duration of 12 months.

Preoperative Serologic Evaluation and Postoperative Serologic Follow-up

In this prospective cohort, preoperative hydatid serology was performed in all included patients using a combination of enzyme-linked immunosorbent assay (ELISA) and indirect hemagglutination assay (IHA). ELISA was performed using the Echinococcus IgG ELISA assay (IBL International GmbH, Tecan Group, Hamburg, Germany), whereas IHA was performed using the ELI.H.A Echinococcus assay (ELITech Microbio, France), according to the manufacturers' instructions. All preoperative and postoperative analyses were conducted in the same laboratory using standardized analytical protocols and the same assay platforms. This standardization ensured longitudinal comparability and minimized analytical variability related to methodological changes.

Postoperative serologic follow-up was scheduled according to a predefined protocol, with testing performed every 1 to 3 months during the first 6 postoperative months, and every 3 to 6 months thereafter, in parallel with clinical and radiological surveillance.

In practice, adherence to this schedule was partially affected by logistical constraints and significantly disrupted during the COVID-19 pandemic, particularly between 12–24 months and 24–36 months, resulting in delayed or missing serologic assessments and imaging follow-up in some patients.

Definitions

Serologic Methods

Enzyme-linked immunosorbent assay (ELISA) refers to an immunoenzymatic method used for antibody detection and quantification, used here to detect IgG antibodies against *Echinococcus granulosus* (5).

Indirect hemagglutination assay (IHA) is a test based on the agglutination of red blood cells coated with hydatid antigens in the presence of specific antibodies (6).

Positive Serology

Serology was considered positive when either of the two tests (ELISA or IHA) exceeded the positivity threshold recommended by the manufacturer.

Seronegativation

For each serologic technique, seronegativation was defined as the first postoperative result falling below the manufacturer-recommended positivity threshold—specifically, an ELISA index < 0.9 for ELISA and a titer < 1:160 for IHA. The date of seronegativation corresponded to the date of the first negative sample.

Statistical Analysis

Data entry and statistical analyses were performed using SPSS version 20.0 and Python version 3.13 with Statsmodels, Pandas, and Matplotlib libraries.

Qualitative variables were described using frequencies and percentages. For quantitative variables, distribution normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests; variables were expressed as mean ± standard deviation or median [interquartile range (IQR)] according to their distribution. Time to seronegativation was calculated from the date of surgery. Patients who did not achieve seronegativation were censored at the date of their last serologic assessment. Seronegativation probabilities were estimated using the Kaplan-Meier method and compared using the log-rank test. Factors associated with time to seronegativation were assessed using univariable and multivariable, Cox regression, with hazard ratios and their 95% confidence intervals.

Longitudinal serologic kinetics were analyzed separately for ELISA and IHA. IHA titers were transformed into $\log_2$ dilution steps, whereas ELISA antibody indices were analyzed on their original scale. Population-level trajectories were explored using locally weighted scatterplot smoothing (LOWESS) with 95% confidence intervals. Two descriptive temporal landmarks were derived from each smoothed trajectory: the maximum fitted value and the first subsequent crossing below the preoperative reference value. These landmarks were used for descriptive purposes and were not considered formally estimated changepoints. Linear mixed-effects models were then fitted to quantify time-related changes in serologic levels, with postoperative time included as a continuous fixed effect and a patient-specific random intercept to account for within-patient correlation. All available measurements were included at their actual assessment times, thereby allowing unequal numbers and irregular timing of observations between patients under a missing-at-random assumption.

All tests were two-sided, with statistical significance set at $p < 0.05$.

Results

Study Population, Cyst Characteristics, and Intraoperative Data

The patient selection flow diagram is presented in Fig. 1. Sixty patients (n = 60) were included in this prospective study, corresponding to 99 hepatic hydatid cysts operated on using a standardized conservative surgical technique. Median follow-up was 61 months (IQR, 36–75). All preoperative demographic, clinical, biological, and cystic characteristics, together with the main intraoperative findings and data, are summarized in Table 1.

Table 1
Cohort Characteristics

Parameter	Result
I. Demographic characteristics	
Number of patients	60
Mean age ± SD (years)	44.07 ± 16.39
Female sex	43 (71.7%)
Rural origin	52 (86.7%)
History of operated HCE	7 (11.7%)
History of operated pulmonary CE	4 (6.7%)
Median postoperative follow-up	61 months [IQR 36–75]
II. Clinical presentation	
Symptomatic patients	54 (90.0%)
Abdominal pain	54 (90.0%)
Tenderness on palpation	35 (60.0%)
Nausea/vomiting	5 (8.3%)
Fever	4 (6.7%)
III. Preoperative laboratory workup	
Hyperleukocytosis	11 (18.3%)
Hypereosinophilia	14 (23.3%)
Abnormal liver function tests	22 (36.7%)
IV. Preoperative hydatid serology	
Seropositive (ELISA and/or IHA)	48 (80.0%)
Dual positivity (ELISA + IHA)	42 (70.0%)
Seronegative (ELISA and IHA)	12 (20.0%)
Positive Western blot (among seronegative)	7/12 (58.3%)
V. Preoperative cyst characteristics	
Median number of cysts/patient	1 [IQR 1–2]
Median cyst size (cm)	7.4 [IQR 5.5–10.7]
Size range (cm)	1.2–19.7

Parameter	Result
I. Demographic characteristics	
WHO stage	CE1: 27 (27.3%) | CE2: 28 (28.3%) | CE3a: 20 (20.2%) | CE3b: 17 (17.2%) | CE4: 7 (7.1%)
VI. Intraoperative data and management of the residual cavity	
Cysts with cystobiliary communication	53 (53.54%)
Omentoplasty associated with drainage	67 cavities (67.67%)

HCE indicates hepatic cystic echinococcosis; CE, cystic echinococcosis; ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; WHO, World Health Organization; IQR, interquartile range; SD, standard deviation.

Postoperative Serologic Evolution

Among the 12 patients who were initially seronegative, 10 (83.3%) remained seronegative throughout follow-up. Two patients experienced an isolated transient ELISA seroconversion, with values exceeding the positivity threshold at 2 months postoperatively before returning below the threshold at 3 and 7 months, respectively. IHA remained negative in these two patients throughout follow-up. No concurrent radiological abnormality was identified, ruling out hydatid recurrence.

Among the 48 patients who were initially seropositive, seronegativation occurred late, with a median of 65 months for ELISA and 71 months for IHA, and more than 60% of patients remained seropositive at 5 years (Fig. 2).

Quantitative analysis of postoperative serologic titers revealed a biphasic immunologic response common to both techniques. An initial transient serologic rebound phase was observed, lasting 3.04 months for IHA, with a peak at 1.52 months postoperatively, and 6.67 months for ELISA, with a later peak at 3.64 months. Beyond this initial phase, a significant linear decline in titers was noted for both techniques; for IHA, the decline began at 3.04 months, with a mean slope of − 2.4 dilutions/month ($p < 0.001$), whereas for ELISA it began at 6.67 months, with a mean slope of − 0.01012/month ($p < 0.001$) (Fig. 3).

Analysis of the annual distribution of postoperative serologic titers showed a concordant evolutionary profile for both techniques. ELISA titers increased during the first 12 months relative to preoperative values, consistent with the serologic rebound phase, then progressively decreased from 12 months onward. A sustained rise was observed between 24 and 36 months in the single patient who presented with hydatid recurrence, whereas two transient re-elevations were noted between 48 and 60 months and between 72 and 84 months in patients who had undergone omentoplasty, without associated radiological abnormality and with a subsequent return to previous values. Similarly, IHA titers showed an initial increase during the first year, followed by a progressive decline throughout follow-up; the only sustained re-elevation also occurred

within the 24- to 36-month interval and corresponded to the case of recurrence confirmed on imaging (Fig. 4).

Predictive Factors of Seronegativation

Regarding predictive factors of seronegativation, univariate analysis found no association between clinical parameters and time to seronegativation (Tables 2 and 3). On biological analysis, among all preoperative parameters assessed, only preoperative C-reactive protein (CRP) was significantly associated with time to seronegativation, for both serologic techniques (ELISA: hazard ratio [HR] = 1.013, 95% CI, 1.002–1.024, p = 0.016; IHA: HR = 1.010, 95% CI, 1.001–1.020, p = 0.032) (Tables 2 and 3).

Table 2
Univariate Analysis of Quantitative Clinical, Biological, and Radiological Cyst Parameters Associated with the 5-Year Seronegativation Rate

Parameter	HR (ELISA)	95% CI (ELISA)	P (ELISA)	HR (IHA)	95% CI (IHA)	P (IHA)
A. Clinical parameter						
Age (years)	0.99	[0.964–1.017]	0.451	0.976	[0.948–1.005]	0.110
B. Biological parameters						
CRP (mg/L)	1.013	[1.002–1.024]	0.016*	1.010	[1.001–1.020]	0.032*
White blood cells	1.000	[1.000–1.000]	0.572	1.000	[1.000–1.000]	0.551
Eosinophils	1.000	[1.000–1.000]	0.513	1.000	[1.000–1.000]	0.279
Total bilirubin	0.998	[0.984–1.013]	0.809	0.981	[0.943–1.021]	0.340
Conjugated bilirubin	1.005	[0.955–1.058]	0.844	0.995	[0.936–1.057]	0.861
AST	1.000	[0.993–1.007]	0.998	0.994	[0.982–1.005]	0.282
ALT	1.000	[0.995–1.004]	0.928	0.997	[0.990–1.004]	0.374
Alkaline phosphatase	1.000	[0.998–1.002]	0.935	1.000	[0.997–1.002]	0.748
GGT	1.000	[0.999–1.001]	0.775	1.000	[0.999–1.001]	0.837
C. Radiological parameters						
Cyst size (cm)	1.081	[0.998–1.171]	0.056	0.996	[0.923–1.076]	0.927
Cyst wall thickness	1.105	[0.703–1.734]	0.666	0.825	[0.533–1.276]	0.387

*HR indicates hazard ratio (Cox model); CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; CRP, C-reactive protein; AST/ALT, aspartate/alanine aminotransferase; GGT, gamma-glutamyltransferase. *P< .05.*

Table 3
Univariate Analysis of Qualitative Clinical and Radiological Cyst Parameters Associated with the 5-Year Seronegativation Rate

Parameter	Subgroup	5-yr seronegativation, ELISA (%)	P	5-yr seronegativation, IHA (%)	P
A. Clinical parameters					
Sex	Male	62.3%	0.630	83.3%	0.759
	Female	50%		54.4%	
Origin	Rural	63.4%	0.699	70.4%	0.425
	Urban	25%		25%	
History of CE	No	56.2%	0.815	63.6%	0.341
	Yes	41.7%		51.4%	
Pain	No	37.5%	0.457	37.5%	0.255
	Yes	55.1%		63.8%	
Nausea/vomiting	No	53.1%	0.263	69.7%	0.673
	Yes	80%		50%	
Fever	No	54%	0.780	62.2%	0.583
	Yes	50%		50%	
B. Radiological parameters					
Exophytic cyst	No	46.7%	0.299	51.3%	0.695
	Yes	60.4%		70.8%	
Hepatic dome involvement	No	57.8%	0.106	75.4%	0.126
	Yes	49.5%		64.6%	
Left hepatic lobe involvement	No	48.1%	0.025*	67.1%	0.611
	Yes	65.2%		78%	
Calcifications	No	53.8%	0.487	71.9%	0.857
	Yes	50%		67.7%	
Daughter vesicles	No	46.5%	0.183	62.5%	0.913
	Yes	64.1%		63.7%	

Parameter	Subgroup	5-yr seronegativation, ELISA (%)	P	5-yr seronegativation, IHA (%)	P
A. Clinical parameters					
Biliary compression	No	52.8%	0.646	73.8%	0.604
	Yes	47.4%		61.9%	
Vascular compression	No	54.7%	0.135	71.8%	0.519
	Yes	48.6%		70.8%	
Cyst content	Rock water	52.8%	0.053	76.2%	0.138
	Bilious	50%		66.7%	
	Purulent	0%		0%	
	Gelatinous	66.7%		66.7%	
CBC	No	34.5%	0.388	45.7%	0.690
	Yes	66.6%		76.1%	
Residual cavity management	Omentoplasty	59.8%	0.503	78.4%	0.068
	Drainage alone	40%		54%	
WHO classification	CE1	52.4%	0.556	73.1%	0.931
	CE2	66.8%		85%	
	CE3a	39.4%		49.1%	
	CE3b	57.1%		75%	
	CE4	0%		50%	

*ELISA indicates enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; CE, cystic echinococcosis; CBC, cystobiliary communication; WHO, World Health Organization. *P < .05.*

Furthermore, patients with initial dual serologic positivity (simultaneously positive ELISA and IHA) achieved seronegativation significantly faster; the 5-year seronegativation rate reached 70.8% in this group, compared with only 33.3% among patients with isolated positivity (p = 0.011) (Fig. 5).

Among cyst characteristics, only left hepatic lobe location was associated with more frequent seronegativation on ELISA (65.2% vs 48.1%, p = 0.025); no other morphological parameter reached statistical significance (Tables 2 and 3).

On multivariate analysis, preoperative CRP and dual serologic positivity (ELISA/IHA) were the only factors independently associated with time to seronegativation (Table 4).

Table 4
Multivariate Analysis of Factors Independently Associated with Time to Seronegativation (Cox Regression)

Independent factor	Technique	Adjusted HR	95% CI	P
Preoperative CRP	ELISA	1.010	[1.004–1.023]	0.004
Preoperative CRP	IHA	1.013	[1.006–1.025]	0.001
Dual ELISA/IHA positivity	IHA	3.7	[1.3–10.5]	0.014

HR indicates hazard ratio; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay.

Discussion

At the conclusion of our study, seronegativation occurred late, with seropositivity persisting in more than 60% of patients at 5 years despite the absence of radiological recurrence. This prolonged delay is explained by a decline of small magnitude—albeit statistically significant—in serologic titers (− 2.4 dilutions/month for IHA and − 0.01012/month for ELISA; $p < 0.001$ for both). Moreover, this decline phase was preceded by an early transient serologic rebound, thereby defining a biphasic postoperative kinetic pattern. On multivariate analysis, preoperative CRP was independently associated with time to seronegativation for both techniques, whereas initial dual ELISA-IHA positivity was associated with faster seronegativation on IHA.

1. Postoperative Serologic Evolution

Rebound effect

The first phase of the biphasic kinetic pattern corresponds to an early serologic rebound. This phenomenon has been described in several prospective series following surgery for cystic echinococcosis, although the observation period varied across studies, ranging from 1 to 10 months (Table 5). This variability should be interpreted with caution, as it likely depends on the frequency of postoperative serologic monitoring and the sampling schedule adopted by each team. In our cohort, this rebound phase was shorter for IHA than for ELISA, lasting 3.04 and 6.67 months, respectively, consistent with published data. Furthermore, two patients in our series had negative preoperative ELISA serology that became positive at 2 months before reverting to negative at 3 and 7 months, respectively, without radiological evidence of recurrence.

Table 5
Summary of Studies Reporting an Early Postoperative Serologic Rebound after Surgery for Cystic Echinococcosis

Author/Year	Study type	Operated patients	Serologic technique	Timing of postoperative serologic rebound
Zarzosa et al., (1999) (8)	Prospective	60	ELISA	1–10 months
Bulut et al., (2001) (15)	Prospective	14	ELISA	1–6 months
Ben Nouir et al., (2008) (7)	Prospective	40	ELISA	1–6 months
Our series	Prospective	60	ELISA	6.67 months
			IHA	3.04 months

ELISA indicates enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay.

Similarly, Ben Nouir et al.(7) reported the occurrence of transient seroconversions among initially ELISA-negative patients, with serology becoming positive in the months following surgery before subsequently reverting to negativity, in the absence of recurrence on imaging. However, the authors did not specify either the exact number of patients affected or the duration of this transient positivity.

Decline phase

Beyond the rebound phase, the literature has generally been limited to reporting seroconversion and persistence of positivity, without quantitative analysis of titer decline over time. The study by Zarzosa et al. (8), conducted in pulmonary cystic echinococcosis, is an exception, describing a biphasic postoperative kinetic pattern without, however, quantifying the decline through slope modeling. This underscores the relevance of our study, which demonstrated a statistically significant linear decline in serologic titers ($p < 0.001$), with a mean slope of − 2.4 dilutions/month for IHA and − 0.01012/month for ELISA.

It should nonetheless be emphasized that, in our cohort, a persistent re-elevation of serologic titers was observed only in the single patient who presented with hydatid recurrence confirmed on imaging. Conversely, two other patients showed transient ELISA elevations without associated radiological abnormality, followed by a spontaneous return to previous values at the subsequent assessment. These observations highlight the importance of distinguishing an isolated, transient serologic fluctuation from a persistent secondary re-elevation occurring after the onset of the decline phase, a finding that should raise suspicion of hydatid recurrence and prompt rapid radiological evaluation.

2. Postoperative Seronegativation: Timing and Predictive Factors

Seronegativation

Historically, postoperative cure was defined by serologic negativation occurring within 2 years after surgery, according to the seminal work of Todorov and Stojanov (9), who, in a prospective cohort of 89 patients

operated on for cystic echinococcosis, observed seronegativation in two-thirds of patients at 2 years and concluded that its absence beyond this interval constituted a sign of recurrence. Subsequent prospective studies have challenged this threshold, demonstrating that the decline of specific antibodies is slower than initially suggested, with seropositivity potentially persisting for up to 5 years in the absence of any documented recurrence on imaging (Table 6). The findings of our series are fully consistent with this perspective, with a median time to seronegativation of 65 months for ELISA and 71 months for IHA, and more than 60% of patients remaining seropositive at 5 years despite the absence of hydatid recurrence.

Table 6
Comparison of Studies Reporting Postoperative Serologic Kinetics

Author/Year	Country/Sample size	Study type	Serologic technique	Serologic persistence	Conclusion
Todorov & Stojanov, (1979) (9)	Bulgaria / 89 operated patients	Prospective, 4-yr follow-up	IHA	Negativation between 6 months and 2 years in 66%	Absence of seronegativation within 2 years is a sign of recurrence.
Wang et al., (2006) (16)	China / 20 operated patients	Prospective, 8-yr follow-up	ELISA	55.6% still positive at 5 years	Persistence or rise in antibody titer beyond 5 years favors recurrence.
Ben Nouir et al., (2008) (7)	Tunisia & Switzerland / 40 young patients	Prospective, 5-yr follow-up	ELISA	57.1% still positive at 5 years	57.1% still positive at 5 years on ELISA.
Eryıldız et al., (2022) (17)	Turkey / 41	Retrospective, 5-yr follow-up	IHA	> 50% of patients remain seropositive beyond 5 years	Mean decrease in IHA titer of −2.6 dilutions in cured patients; mean increase of + 3.4 dilutions in recurrence.
Our series	Tunisia — 60 patients (99 cysts)	Prospective, median follow-up 61 months	ELISA / IHA	Median time to seronegativation: 65 months (ELISA), 71 months (IHA)	More than 60% of patients still seropositive at 5 years.

IHA indicates indirect hemagglutination assay; ELISA, enzyme-linked immunosorbent assay.

Predictive factors of seronegativation

To date, no published study has specifically evaluated the factors influencing time to seronegativation in HCE. In our cohort, multivariate analysis showed that preoperative CRP and dual serologic positivity (ELISA/IHA) were the only factors independently associated with time to seronegativation, whereas no other clinical or morphological parameter provided independent predictive value.

3. Immunological Basis

From an immunological standpoint, antibody kinetics can be explained by the existence of two compartments of antibody-secreting cells. The early response is dominated by an extrafollicular wave of short-lived plasmablasts and plasma cells, responsible for the initial serologic peak. In parallel, a fraction of antibody-secreting cells migrates to the bone marrow and differentiates into long-lived, post-mitotic plasma cells capable of sustaining prolonged antibody production (10).

The prolonged persistence of seropositivity may therefore primarily reflect the longevity of long-lived plasma cells and the persistence of immunologic memory, rather than residual parasitic activity or recurrence.

In this context, analysis of immunoglobulin subclasses has been proposed as a potential approach for distinguishing cured patients from cases of recurrence. However, few studies have specifically explored this avenue. The first notable contribution dates back to the study by Riganò et al. (11), whose objective was not to establish a direct correlation with cyst viability but rather to compare immunologic profiles according to treatment response. The authors notably observed a higher proportion of IgG3-positive patients and a lower proportion of IgG4-positive patients among good responders, with the inverse pattern observed among poor responders.

This observation subsequently guided more structured research, notably the study by Tenguria et al.(12), which more systematically evaluated IgG subclasses during postoperative follow-up. The authors demonstrated a significant decrease in IgG1, IgG2, and particularly IgG4 responses (IgG1, 82% to 46%; IgG2, 74% to 34%; IgG4, 86% to 28%), with high diagnostic performance for IgG4 (sensitivity 86%, specificity 98.33%, accuracy 92.72%). Thus, immunologic memory may be expressed differentially across IgG subclasses, with some persisting longer than others during follow-up, IgG3 persisting preferentially in good responders and IgG4 in poor responders. These findings suggest that the study of IgG subclasses, particularly IgG4, could represent a complementary tool for refining the interpretation of persistent postoperative seropositivity and for better distinguishing immunologic memory from parasitic activity.

The serologic rebound effect observed after surgery may reflect a secondary humoral response induced by the release of hydatid antigens during cyst manipulation (7,14). This acute antigenic stimulation could preferentially reactivate the extrafollicular pathway, leading to a transient expansion of plasmablasts and short-lived plasma cells. After surgery, the disappearance of antigenic stimulation would result in a rapid contraction of this response, accounting for the subsequent decline in antibody titers.

This hypothesis could also explain our predictive factors of seronegativation. Elevated preoperative CRP, reflecting a complicated cyst, and dual ELISA-IHA positivity may serve as indirect markers of more intense antigenic stimulation, favoring a humoral response dominated by the short-lived plasma cell compartment. Once the antigenic source is removed, this response would resolve rapidly, resulting in earlier seronegativation.

Data from the literature support these hypotheses, showing that the intensity of the serologic response increases when cyst integrity is compromised and antigenic exposure becomes greater. In the study by Todorov et al. (13), complicated cysts (suppurated, with pericystic inflammation) showed markedly higher

serologic titers, overall approximately 2 to 3 times higher than those observed in cysts with clear fluid content. Consistent with these findings, Caushi al.(14) reported, among 362 patients, a positive IgG ELISA in 50% of cases (181/362), with significantly higher sensitivity in complicated cysts (70%, 175 patients) compared with uncomplicated cysts (18.8%, 21 patients).

Limitations

This study has several limitations, including its single-center design, limited sample size, and absence of a control group. In addition, the occurrence of a single recurrence limits diagnostic evaluation of serologic variations.

Although a predefined postoperative follow-up schedule was established, adherence was partially affected by real-world constraints, including healthcare accessibility limitations and disruptions during the COVID-19 pandemic. These deviations were non-systematic and did not appear to be related to clinical outcomes. Serologic assessments were delayed rather than systematically missing, thereby limiting the risk of selection bias while preserving the validity of longitudinal trends.

Conclusions

The decline in serologic titers after surgery is significantly slow, accounting for the persistence of seropositivity in more than 60% of patients at 5 years. An isolated positive serology should therefore not be interpreted alone as indicative of recurrence. Conversely, a persistent secondary re-elevation of serologic titers following the postoperative rebound should raise suspicion of recurrence and prompt clinical and radiological re-evaluation.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board (Comité de Protection des Personnes) of Habib Bourguiba University Hospital, Sfax, Tunisia. Written informed consent to participate was obtained from each patient prior to inclusion. All procedures were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments. The trial was registered in the Pan African Clinical Trial Registry under the number PACTR202608878713534.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Aymen Trigui: Study conception and design, management of the study protocol, data collection, data analysis, and writing of the main manuscript text.

Ahmed Hadj Taieb: Writing of the main manuscript text and manuscript review.

Youssef Mejdoub: Data collection and clinical management.

Yosra Mejdoub: Statistical analysis and interpretation of data.

Rami Zouari: Data collection and clinical management.

Majdi Kchaou: Data collection and clinical management.

Sami Fendri: Data collection and clinical management.

Mohammad Saad Saumtally: Data collection and clinical management.

Rafik Mzali: Management of the study protocol and manuscript review.

Imen Kharrat: Data collection and parasitological assessment.

Ali Ayadi: Organization and coordination of the study.

Chadli Dziri: Manuscript review and scientific supervision.

Saleh Boujelbene: Organization and coordination of the study and manuscript review.

Mohamed Ben Amar: Data analysis, interpretation of results, and organization of the study.

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Not applicable.

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Figures

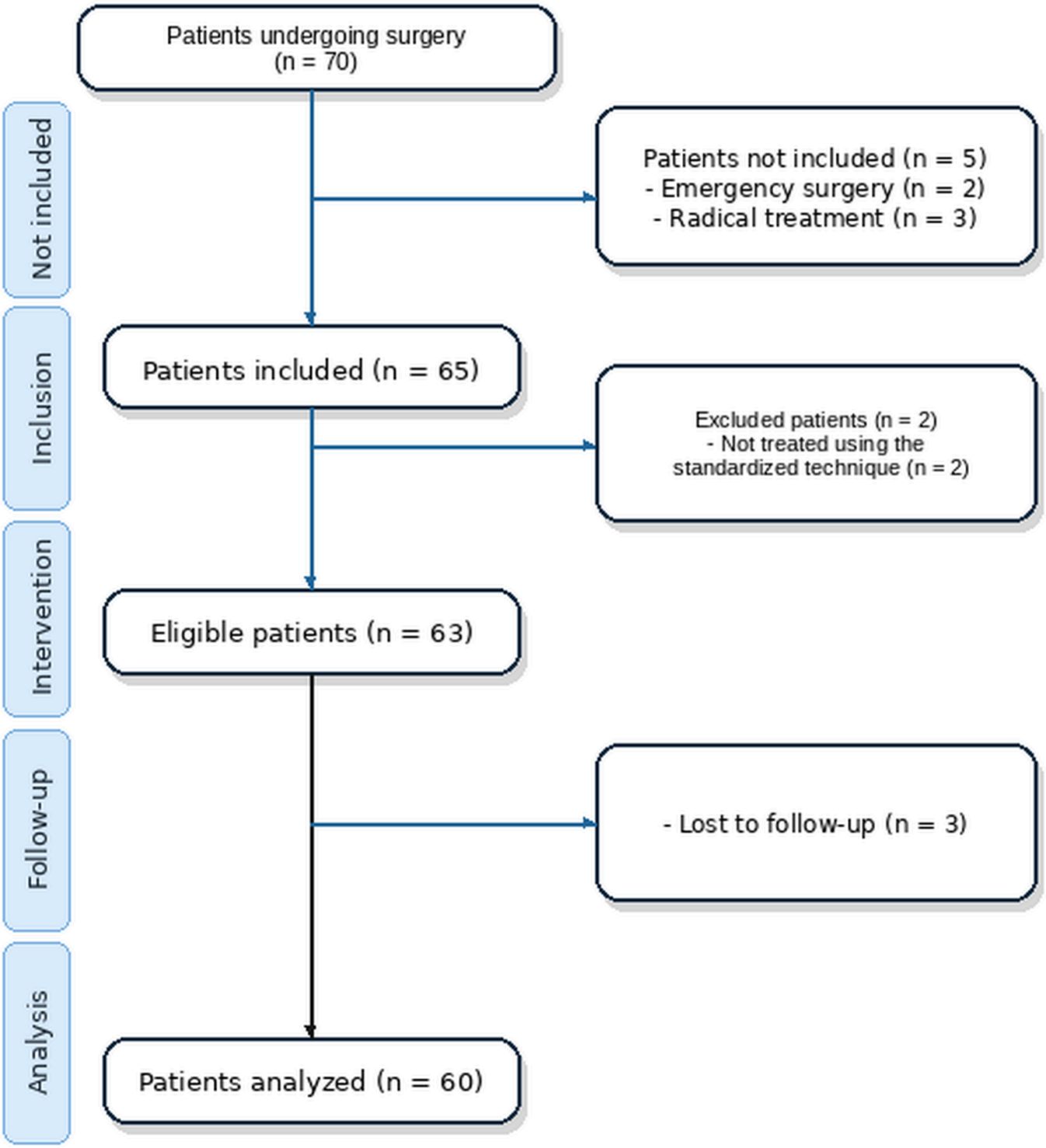


Figure 1

Flow diagram of study population selection.

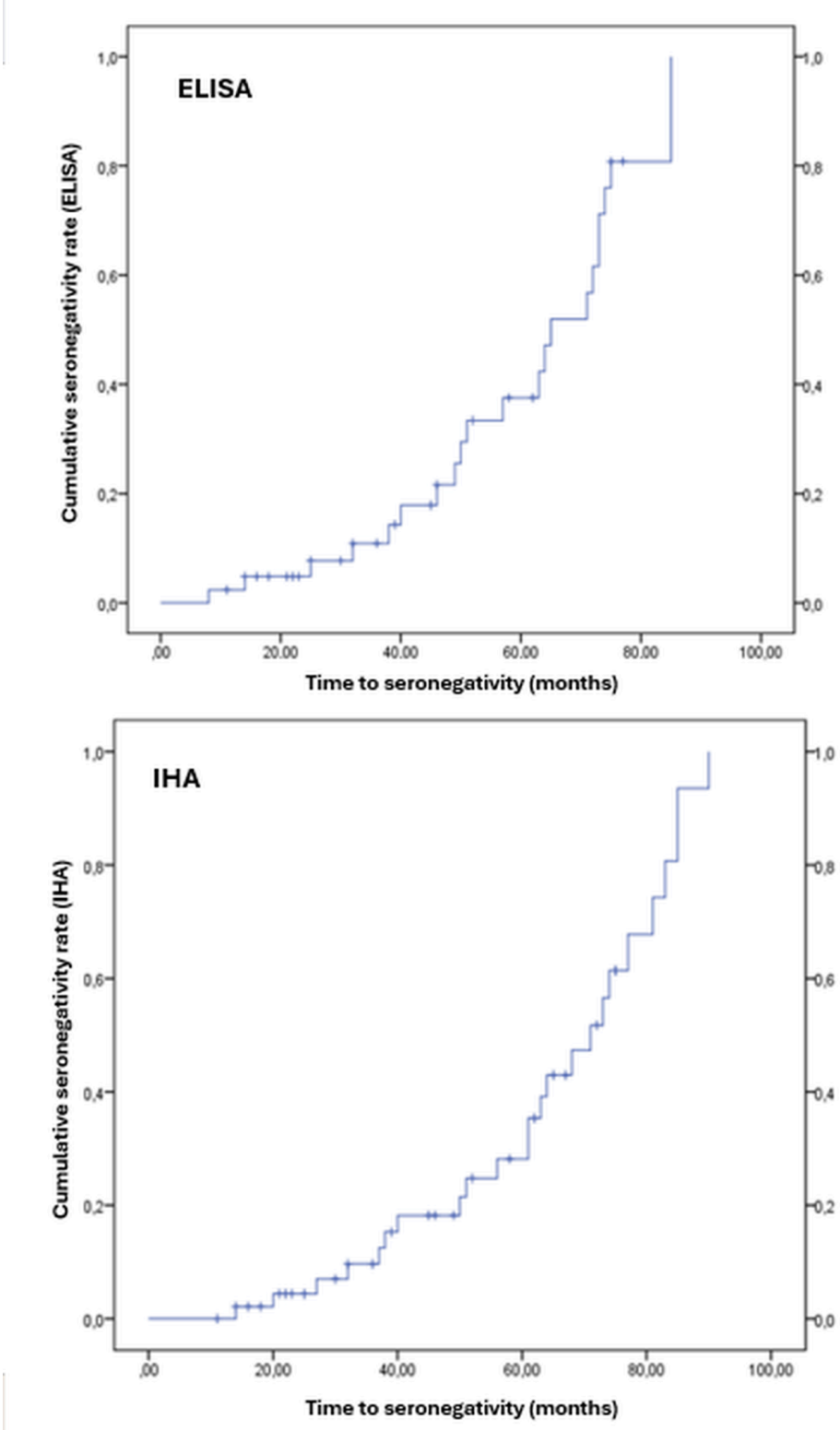


Figure 2

Time to seronegativation according to ELISA and IHA technique.

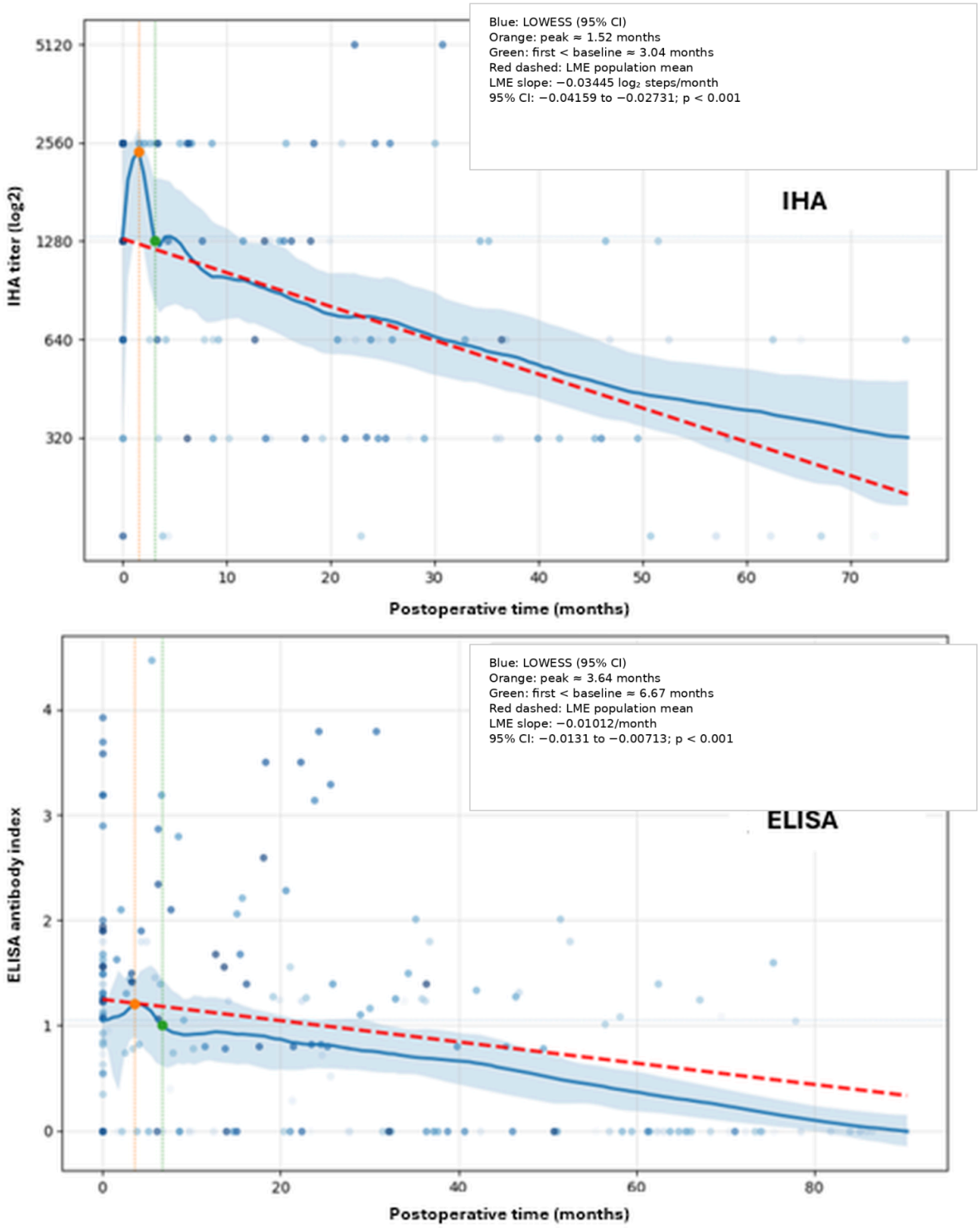


Figure 3

Postoperative serologic kinetics.

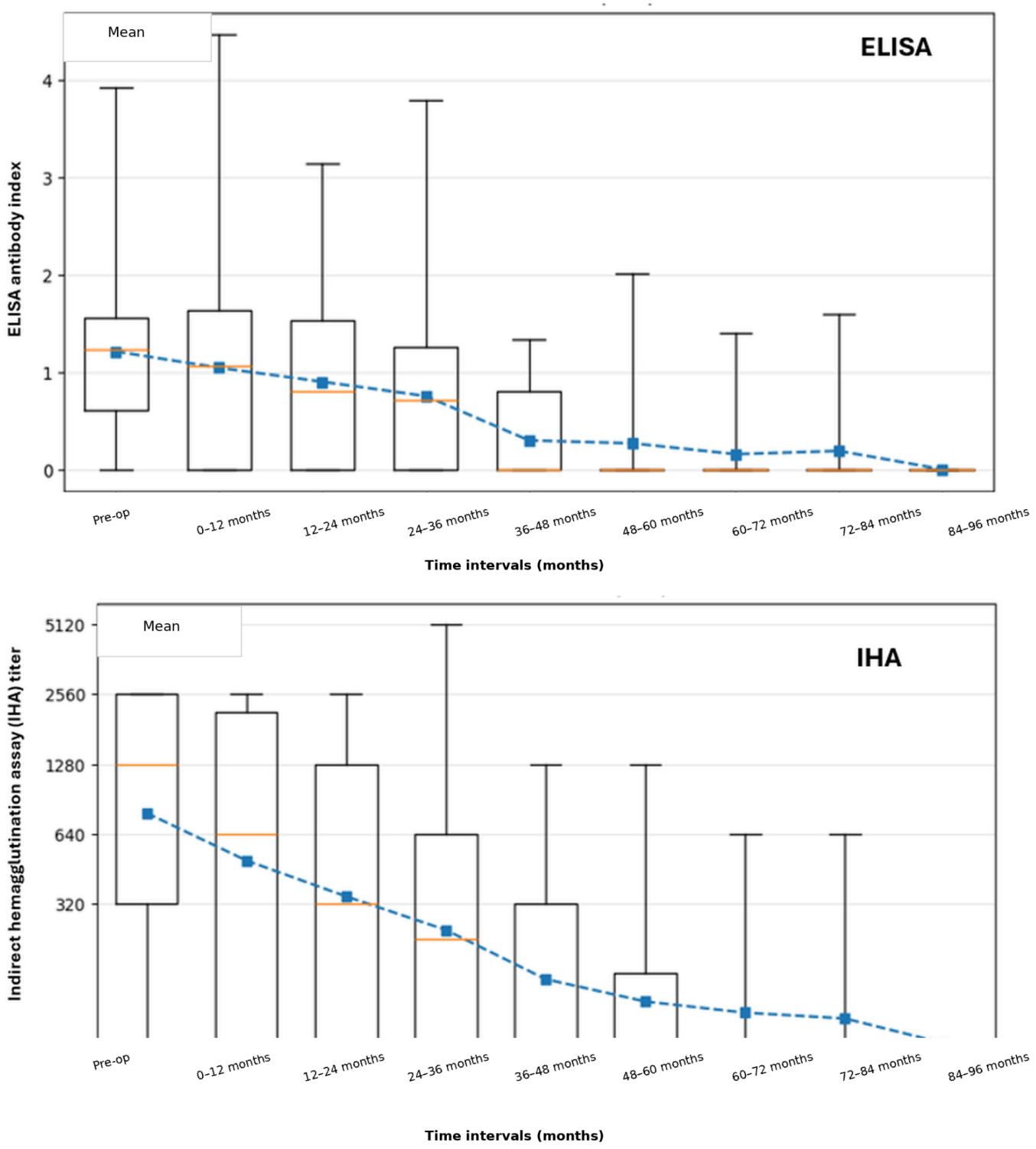


Figure 4

Annual distribution of postoperative ELISA and IHA titers.

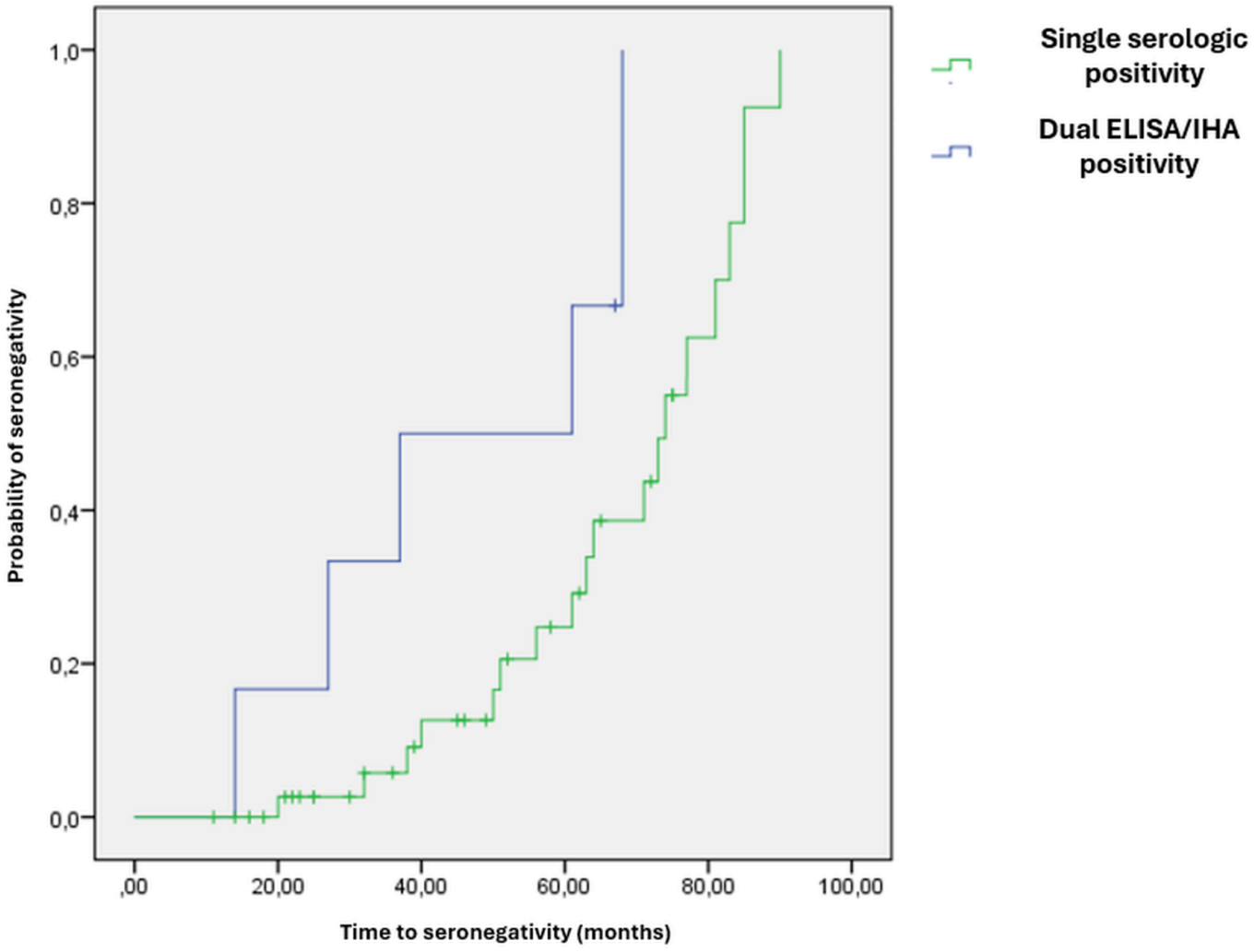


Figure 5

Time to seronegativation according to the presence or absence of dual ELISA-IHA positivity.