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Original Article

Treatment outcomes for chronic limb-threatening ischemia and identification of predictive factors for poor outcomes

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Abstract

Aim: Chronic limb-threatening ischemia (CLTI), the advanced stage of lower extremity peripheral artery disease (PAD), is associated with high amputation rates, mortality, and diminished quality of life. This study aimed to evaluate treatment outcomes and identify independent predictors of poor outcomes in these patients.

Material and Methods: This retrospective observational analytic study included CLTI patients (rest pain/tissue loss with absolute ankle pressure <50 mmHg) between January 2022 and March 2025 at Latakia University Hospital. Patients were categorized into interventional, deferred interventional, and conservative groups. Primary outcomes were 1-year amputation-free survival (AFS) and overall survival (OS).

Results: The sample comprised 152 patients (mean age: 65.28±8.5 years); 47.4% underwent intervention within 6 weeks, 27.6% received conservative management, and 25.0% had deferred intervention after 6 weeks. No statistically significant differences occurred among groups regarding 1-year AFS (P = 0.60) or OS (P = 0.30). Interventional type (open vs. endovascular) did not significantly affect AFS or OS. Significant independent predictors affecting both AFS and OS were age, heart disease, chronic obstructive pulmonary disease (COPD), previous revascularization, and anemia. Moderate to severe renal failure independently predicted OS only.

Conclusion: No significant outcome differences were detected among treatment strategies. Deferred intervention is feasible in selected patients without worsening short-term outcomes. Though not replacing indicated revascularization, conservative management may represent a reasonable approach yielding favorable 12-month outcomes in carefully selected patients. CLTI outcomes heavily depend on patient comorbidities and overall condition rather than solely limb clinical/anatomical staging. Age, heart disease, COPD, previous revascularization, anemia, and moderate to severe renal failure are crucial predictors of poor outcomes.

Keywords: Chronic limb-threatening ischemia, interventional treatment, deferred interventional treatment, conservative management, amputation-free survival

INTRODUCTION

Chronic limb-threatening ischemia (CLTI) is the end stage of peripheral artery disease (PAD), defined as PAD with rest pain, gangrene, or lower limb ulceration >2 weeks [1]. CLTI affects 11% of PAD patients and is associated with high limb loss rates, mortality, care costs, and reduced quality of life [1,2]. Approximately 20% of CLTI patients undergo amputation within one year [3]. One-year mortality ranges from 20% to 25%, especially in those with advanced comorbidities, tissue loss, or ineligibility for revascularization [4]. Repeat revascularizations are often needed due to progressive atherosclerosis and complex anatomy [5]. While all patients should receive guideline-directed medical therapy, restoring inline flow is frequently necessary to prevent limb loss [6]; the Global Vascular Guidelines propose a structured approach to revascularization based on the assessment of Patient risk, Limb severity, and Anatomic complexity (PLAN) [1]. Some clinical stages experience longer delays, which reflects greater comorbidity or frailty and the need for preoperative

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optimization [7]. In addition, a selective, staged approach may be safe and effective in appropriately selected low- to moderate-risk CLTI patients [8]. Recently, conservative management has emerged as an appropriate option for patients unsuitable for revascularization due to complex arterial lesions or high surgical risk from comorbidities [9]. CLTI is a central research priority in vascular surgery; a multidisciplinary agenda is essential, with key endpoints including limb salvage, survival, quality of life, and avoiding unnecessary interventions [10]. However, the optimal timing of revascularization and the role of preoperative medical optimization remain poorly defined. Therefore, this study aims to investigate the impact of revascularization (early or delayed) versus conservative management on amputation-free survival (AFS) and overall survival (OS) in CLTI patients. These outcomes represent hard clinical endpoints in CLTI management, as they reflect the clinical benefit to the patient rather than merely the technical success of the procedure. Consequently, this study focuses on identifying predictive factors for poor outcomes, in order to improve preoperative risk stratification and guide treatment decisions toward the most effective solutions for each patient.

MATERIAL AND METHODS

This retrospective observational analytic study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines [11]. While the STROBE-Equity extension was considered, data on equity-related variables (socioeconomic status, education, race/ethnicity, language, and place of residence) were not routinely available in the clinical database, reflecting limitations of routine clinical data collection. Future prospective studies should prioritize the collection of these variables to enable health equity analyses.

Ethics statement:

This retrospective study was conducted at Latakia University Hospital. The study protocol was reviewed and approved by the Institutional Ethics Committee of the Faculty of Medicine at Latakia University (Council Approval No: 1533; Approval Date: May 27, 2025). Due to the retrospective design of the study and the use of anonymized clinical data, the requirement for informed consent was waived by the committee. All procedures were conducted in accordance with the Declaration of Helsinki.

Patient selection

This retrospective study included consecutive eligible patients diagnosed with CLTI who presented between January 2022 and March 2025 and did not undergo primary major amputation. Patients were identified through review of vascular laboratory data and medical charts. Inclusion criteria were: (1) the presence of ischemic rest pain or tissue loss, and (2) a systolic ankle pressure < 50 mmHg. Patients who met only one of these criteria (e.g., low ankle pressure without rest pain or tissue loss) were excluded. Exclusion criteria included acute limb ischemia, Buerger's disease, vasculitis, and patients who received treatment for CLTI at another institution. Due to the retrospective nature of data collection and the pre-specification of the exclusion criteria, a detailed patient flow diagram with exact exclusion numbers and specific reasons for exclusion could not be generated; however, the inclusion and exclusion criteria were consistently applied to ensure a homogeneous study cohort.

Data Collection and Outcome Measures

Patient characteristics (age, sex, smoking, comorbidities: diabetes mellitus, moderate to severe renal failure, chronic obstructive pulmonary disease (COPD), heart disease, hypertension, dyslipidemia, anemia, Fontaine stage III/IV and previous revascularization) were extracted from clinical and operative records. Smoking status: current/former vs. never. Hypertension, diabetes mellitus, dyslipidemia: diagnosed by physician or on treatment. Anemia: World Health Organization (WHO) criteria (Hemoglobin < 13 g/dL for men, < 12 g/dL for women) [12]. Heart disease: history of myocardial infarction (MI), angina, heart failure, or prior coronary revascularization. Moderate to severe renal failure: Kidney Disease: Improving Global Outcomes (KDIGO) stages G3-5 [13]. COPD confirmed by pulmonologist. Fontaine stage III: presence of ischemic rest pain, Fontaine Stage IV: presence of ulceration or gangrene. Previous Revascularization was defined as a history of any prior surgical or endovascular intervention performed to restore blood flow to the same limb. Follow-up data included limb salvage and survival. Patients were grouped as: "Interventional treatment" (revascularization ≤ 6 weeks), "deferred interventional treatment" (> 6 weeks after conservative management), or "permanently conservative management" (no revascularization between diagnosis and death/last follow-up). A 6-week threshold was pragmatically selected to distinguish early from delayed interventional treatment, as this timeframe may, in some patients, represent a planned strategy to permit preoperative medical optimization, address active comorbidities, control infection, complete necessary diagnostic evaluations before intervention, and monitor the response to conservative management. Data collected until last follow-up (up to 1 year) or death. Missing follow-up data obtained by contacting patients/family physicians. Primary outcome: AFS = time from diagnosis to death or major amputation (proximal to ankle). OS also assessed. Secondary reinterventions on the ipsilateral limb or new contralateral procedures were not counted as new cases.

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Statistical analyses

This observational retrospective analytic study summarized quantitative variables as means and standard deviations, and qualitative variables as frequencies and percentages. Continuous variables were compared among the three independent groups using one-way analysis of variance (ANOVA), and categorical variables using the chi-square test. AFS and OS were estimated with Kaplan–Meier curves, and differences between curves were assessed with the log-rank test. Univariate Cox regression was performed to screen candidate variables. Variables that were statistically significant in univariate analysis, together with clinically relevant variables, were entered into a multivariable Cox proportional hazards regression model to identify independent predictors of poor outcomes. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. Statistical significance was defined as $P < 0.05$. Normality of continuous variables was assessed using the Shapiro–Wilk test. Homogeneity of variances was verified using Levene's test. The proportional hazards assumption for Cox regression was assessed using Schoenfeld residuals. All analyses were conducted using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Distribution of the sample

The study sample comprised 152 patients diagnosed with CLTI who met the inclusion criteria, with a mean age of 65.28±8.5 years. The sample was predominantly male (77%), and the majority were smokers (67.8%). The most prevalent comorbidity was diabetes mellitus (59.2%), followed by hypertension (58.6%), while the least common was moderate to severe renal failure (12.5%). The clinical characteristics of the study groups are presented in Table 1 (Baseline demographic and clinical characteristics of the study cohort across the three treatment groups). The majority of the sample (47.4%) underwent interventional treatment within 6 weeks; 27.6% received conservative management, and 25.0% underwent deferred interventional treatment after 6 weeks. Figure 1 presents the treatment flowchart. Of the interventional procedures, 75.5% were performed by open surgery and 24.5% by endovascular surgery. No statistically significant differences were observed in the distribution of baseline demographic and clinical variables among the three study groups ($P > 0.05$ for all comparisons). As shown in Table 1 (Baseline demographic and clinical characteristics of the study cohort across the three treatment groups).

Table 1. Baseline demographic and clinical characteristics of the study cohort across the three treatment groups

Characteristics		Study sample			P
		Interventional (n = 72)	Deferred interventional (n = 38)	Conservative (n = 42)	
Sex	Male	58 (80.6%)	30 (78.9%)	29 (69.0%)	0.30
	Female	14 (19.4%)	8 (21.1%)	13 (31.0%)	
Age		65.1 ± 9.1	66.8 ± 7.3	68.1 ± 7.4	0.16
Smoking		53 (73.6%)	26 (68.4%)	24 (57.1%)	0.10
Diabetes mellitus		41 (56.9%)	23 (60.5%)	26 (61.9%)	0.80
Hypertension		40 (55.6%)	26 (68.4%)	23 (54.8%)	0.30
COPD		27 (37.5%)	13 (34.2%)	10 (23.8%)	0.30
Heart disease		26 (36.1%)	10 (26.3%)	12 (28.6%)	0.50
Moderate to severe renal failure		6 (8.3%)	6 (15.8%)	7 (16.7%)	0.30
Anemia		26 (36.1%)	16 (42.1%)	13 (31.0%)	0.50
Dyslipidemia		19 (26.4%)	16 (42.1%)	12 (28.6%)	0.20
Previous revascularization		20 (27.8%)	6 (15.8%)	11 (26.2%)	0.30
Fontaine stage	Stage III	39 (54.2%)	20 (52.6%)	20 (47.6%)	0.70
	Stage IV	33 (45.8%)	18 (47.4%)	22 (52.4%)	

Data are presented as mean ± standard deviation or number (percentage); COPD: chronic obstructive pulmonary disease

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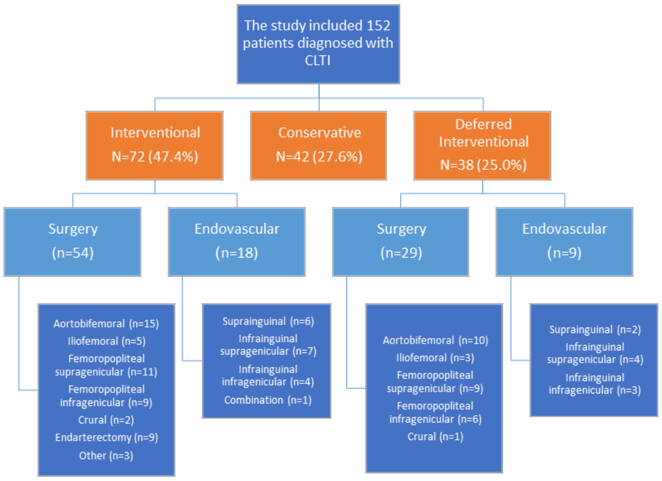

Figure 1. The treatment flowchart of patients with CLTI

Amputation-free and overall survival

At one year, AFS rates were 63.3% in the interventional group, 68.6% in the deferred interventional group, and 62.5% in the conservative management group. Component analysis of the composite endpoint at 12 months revealed that major amputation was the primary component event driving the composite endpoint across all groups. As shown in Table 2 (Amputation-free survival during the follow-up time intervals). Corresponding OS rates were 84.1%, 86.4%, and 75.1%, respectively. As shown in Table 3 (Overall survival during the follow-up time intervals). Although AFS (P = 0.60) and OS (P = 0.30) showed no statistically significant differences among the three groups, both rates were numerically higher in the deferred interventional group, while the conservative management group had the lowest rates. Figure 2 (Kaplan–Meier curves for amputation-free survival). Figure 3 (Kaplan-Meier curves for overall survival). The type of interventional treatment (open vs. endovascular surgery) had no statistically significant effect on either AFS (P = 0.80) or OS (P = 0.50). The most significant independent predictors affecting AFS and OS were age, heart disease, COPD, previous revascularization, and anemia. Additionally, moderate to severe renal failure was an independent predictor associated with OS only. As shown in Table 4 (Independent predictors associated with amputation-free survival) and Table 5 (Independent predictors associated with overall survival).

Table 2. Amputation-free survival during the follow-up time intervals

Management	Months from CLTI diagnosis				
Interventional	**0**	**3**	**6**	**9**	**12**
Patients at risk	72	65	61	55	41
Number of events					
Major amputation	0	3	4	7	15
Death	0	4	5	7	9
Amputation-free survival %	100.0%	90.3%	86.1%	80.2%	63.3%
Deferred Interventional	**0**	**3**	**6**	**9**	**12**
Patients at risk	38	38	35	32	23
Number of events					
Major amputation	0	0	2	2	8
Death	0	0	1	2	3
Amputation-free survival %	100.0%	100.0%	92.1%	89.3%	68.6%
Conservative	**0**	**3**	**6**	**9**	**12**
Patients at risk	42	38	34	27	24
Number of events					
Major amputation	0	3	4	5	8
Death	0	1	3	7	7
Amputation-free survival %	100.0%	90.5%	83.1%	70.4%	62.5%

CLTI: chronic limb-threatening ischemia; Events include major amputation or death; Data are cumulative for each time interval

Table 3. Overall survival during the follow-up time intervals

Management	Months from CLTI diagnosis				
Interventional	**0**	**3**	**6**	**9**	**12**
Patients at risk	72	65	64	58	50
Number of events (death)	0	7	8	9	11
Overall survival %	100.0%	90.3%	88.9%	87.4%	84.1%
Deferred Interventional	**0**	**3**	**6**	**9**	**12**
Patients at risk	38	38	35	32	30
Number of events (death)	0	0	3	4	5
Overall survival %	100.0%	100%	92.1%	89.3%	86.4%
Conservative	**0**	**3**	**6**	**9**	**12**
Patients at risk	42	38	35	28	28
Number of events (death)	0	4	6	10	10
Overall survival %	100.0%	90.5%	85.6%	75.1%	75.1%

CLTI: chronic limb-threatening ischemia; Events include death; Data are cumulative for each time interval

Table 4. Independent predictors associated with amputation-free survival in patients with chronic limb-threatening ischemia

Characteristics		Univariate analysis			Multivariate analysis		
		HR	CI 95%	P	HR	CI 95%	P
Sex		0.8	0.3–1.8	0.50		-	
Age (per 10-year increase)		2.4	1.4–4.1	0.002	1.9	1.1–3.3	0.021
Smoking		0.7	0.2–2.4	0.70		-	
Previous revascularization		2.7	1.7–4.3	0.001	2.0	1.1–3.6	0.015
Moderate to severe renal failure		1.3	0.6–2.2	0.07		-	
Diabetes mellitus		0.9	0.3–2.4	0.80		-	
Heart disease		3.0	1.4–6.3	0.001	2.5	1.1–5.5	0.028
COPD		2.6	1.2–5.6	0.015	2.8	1.8–4.5	0.004
Hypertension		2.4	1.5–3.7	0.001	0.8	0.2–3.3	0.80
Dyslipidemia		1.7	0.9–3.4	0.06		-	
Anemia		2.1	1.4–3.4	0.003	2.6	1.2–5.5	0.015
Fontaine stage	Stage III	Ref.	-			-	
	Stage IV	2.2	1.5–3.4	0.02	1.0	0.2–5.1	0.60
Management	Conservative	Ref.	-				
	Interventional	1.1	0.5–2.5	0.60		-	
	Deferred Interventional	0.9	0.3–2.4	0.80			

HR: hazard ratio; CI: confidence interval; COPD: chronic obstructive pulmonary disease; Ref.: reference category

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Table 5. Independent predictors associated with overall survival in patients with chronic limb-threatening ischemia

Characteristics		Univariate analysis			Multivariate analysis		
		HR	CI 95%	P	HR	CI 95%	P
Sex		0.9	0.3–2.7	0.85		-	
Age (per 10-year increase)		2.5	1.2–5.2	0.014	2.0	1.1–4.0	0.024
Smoking		0.8	0.3–2.1	0.65		-	
Previous revascularization		2.1	1.1–4.4	0.025	1.8	1.0–3.2	0.050
Moderate to severe renal failure		2.3	1.1–4.2	0.018	2.9	1.6–5.4	0.001
Diabetes mellitus		1.1	0.4–3.0	0.85		-	
Heart disease		3.5	1.6–7.6	0.001	3.4	1.6–7.4	0.020
COPD		1.7	1.1–2.7	0.022	1.4	1.0–1.9	0.038
Hypertension		1.9	0.9–4.0	0.09		-	
Dyslipidemia		1.0	0.4–2.5	0.99		-	
Anemia		2.3	1.1–4.8	0.027	2.3	1.1–4.3	0.027
Fontaine stage	Stage III	Ref.	-	-		-	
	Stage IV	1.6	0.8–3.2	0.18			
Management	Conservative	Ref.	-	-			
	Interventional	1.5	0.8–2.7	0.18		-	
	Deferred Interventional	1.3	0.6–2.6	0.46			

HR: hazard ratio; CI: confidence interval; COPD: chronic obstructive pulmonary disease; Ref.: reference category

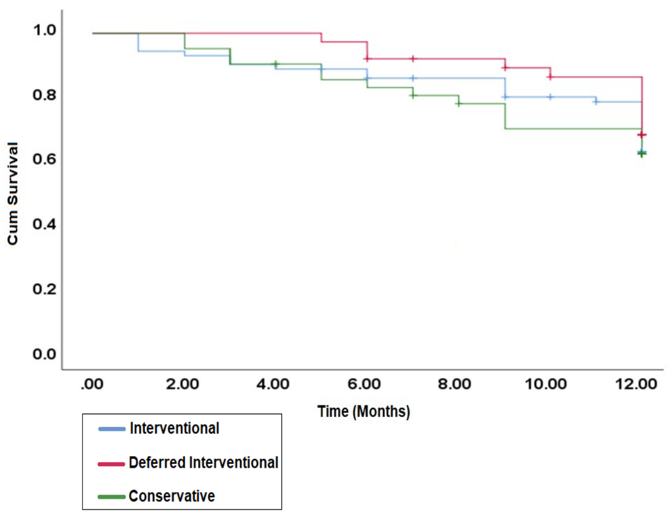

Figure 2. Kaplan–Meier curves for amputation free survival; Interventional (blue line), Deferred Interventional (red line), and Conservative (green line); Vertical ticks (+) indicate censored patients

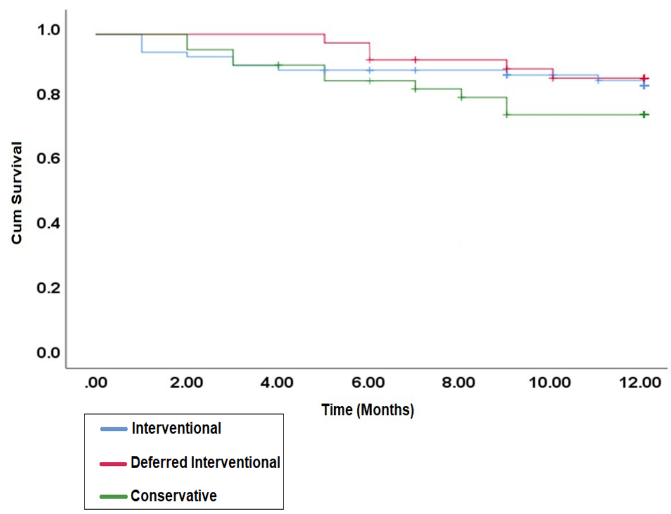

Figure 3. Kaplan-Meier curves for overall survival; Interventional (blue line), Deferred Interventional (red line), and Conservative (green line); Vertical ticks (+) indicate censored patients

DISCUSSION

Our study results, which showed no statistically significant differences in AFS and OS among the treatment groups, are consistent with previous and recent international studies reaching the same conclusion [8,14]. This balance in the final outcomes may reflect that individualizing the treatment decision based on each patient's clinical status and baseline characteristics helped optimize outcomes within each respective pathway, thereby achieving comparable 12-month survival profiles without demonstrating an absolute superiority of one treatment modality over another. Despite this numerical similarity at 12 months, a variation is observed in the distribution of events and AFS rates across the interim follow-up periods (3, 6, and 9 months). This may be clinically attributable to multiple factors, including the severity of the initial limb ischemia or its stability, as well as differences in baseline patient characteristics and comorbidities at the time of study enrollment. Moreover, the deferred interventional group showed numerically better survival rates in several studies [8,14]. The effect of delay on outcomes of interventional treatment continues to be an area of interest in contemporary research. A recent study demonstrated that 81% of experts endorsed delaying intervention when it could alter the treatment plan [15].

Furthermore, our findings, along with those of global studies showing that the burden of comorbidities is the main driver of mortality, with neither treatment modality nor clinical stage of the limb having statistical significance, support the concept of a planned delay for preoperative clinical optimization and comorbidity management to improve prognosis [16]. The safe window may differ across studies; in this cohort, delayed intervention beyond six weeks was not associated with significantly worse outcomes. Nevertheless, these studies suggest that carefully selected patients may tolerate delayed intervention without clearly worse short-term outcomes [17], and may even positively influence interventional outcomes after necessary workup and preparation. This finding may help surgeons consider delay strategies and move away from the notion that early intervention is the best option for all patients.

The OS profiles observed across our study pathways are broadly consistent with contemporary literature. A 2024 study with a similar methodology reported a one-year survival rate of 81.9% in the CLTI subgroup [18], while a more recent analysis of endovascularly treated CLTI patients found one-year survival outcomes that align closely with our findings [19]. Collectively, these comparable survival estimates reinforce the external validity of our results and suggest that the survival outcomes achieved across all three treatment strategies in our center are within the range reported by other high-volume limb preservation programs.

The outcomes of conservative management may reflect a subset of patients with low-risk disease who do not require urgent revascularization, but they may also include patients with high frailty or non-reconstructible arterial disease. Therefore, the results of our study remain valuable, demonstrating that conservative management may represent a reasonable management approach in carefully selected patients who are unsuitable for or do not require immediate revascularization and was associated with acceptable outcomes in selected patients within this cohort. This is also supported by a systematic review of multiple studies on conservative management in CLTI, which reported rates similar to ours [20].

We found no statistically significant differences in AFS between open surgery and endovascular treatment. This reinforces the concept of individually tailored treatment without superiority of one technique over the other; the decision should be based on a structured assessment of patient risk, limb severity, and anatomic complexity [21]. This individualized decision must take into account several variables that were strongly associated with AFS and OS in our study and similar studies [8,14,22], such as age, heart disease, COPD, previous revascularization, and anemia. In addition, moderate to severe renal failure was a major driver of mortality, often occurring before patients underwent amputation.

Study limitations

Several limitations should be acknowledged. The retrospective design and reliance on routine clinical records may introduce incomplete documentation and selection bias. The study was conducted in a country without universal health insurance, and many patients lacked adequate coverage. This financial barrier, along with logistical constraints—such as intermittent unavailability of endovascular interventions due to equipment failures, limited bed and intensive care unit capacity, operating room availability, and patient preferences shaped by economic and personal circumstances—contributed to the predominance of surgical over endovascular revascularization and prevented the performance of hybrid procedures. Importantly, delays in revascularization and the choice of conservative management were not solely due to negative factors. In some patients, delayed intervention reflected a planned strategy for preoperative optimization, comorbidity management, infection control, and diagnostic workup. Similarly, conservative management was chosen based on clinical judgment, anatomical unsuitability for revascularization, or patient preferences informed by realistic risk-benefit expectations. However, the lack of a standardized protocol for conservative medical optimization during the deferred intervention period, or when conservative management is applied permanently, may have influenced the outcomes of these two groups. Treatment allocation was based on clinical judgment, anatomical suitability, comorbidity burden, and logistical factors, not random assignment, introducing confounding by indication. This interplay of factors—both positive (planned) and negative (financial/logistical)—complicates the interpretation of treatment allocation and outcomes and may introduce selection bias. Therefore, generalizability to healthcare systems with universal coverage, different financial structures, or consistent endovascular availability may be limited. Data on key health equity characteristics (socioeconomic status, education, race, ethnicity, language proficiency, residence) were not systematically captured, precluding disparity analysis. The study shows no statistically significant differences in the distribution of baseline characteristics among the three groups. However, given the retrospective design, no formal sample size calculation was performed, which may have resulted in a limited sample size—particularly in the deferred intervention group. This may have reduced the statistical power to detect small but potentially clinically relevant differences among the treatment groups. Additionally, despite adjustment for relevant confounders, residual confounding by clinically relevant unmeasured variables—such as atrial fibrillation, poor glycemic control, and graft occlusion—remains a possibility. Finally, due to the retrospective nature of this study and the extended recruitment period, detailed data on the exact number of screened patients and specific reasons for exclusion were not systematically recorded in the clinical database. However, the exclusion criteria—acute limb ischemia, Buerger's disease, vasculitis, and treatment at another institution—were clearly predefined and consistently applied during the initial chart review, regardless of the availability of detailed screening logs. Consequently, we were unable to construct a comprehensive patient flow diagram with precise exclusion numbers. This limitation, inherent to the retrospective design, limits the transparency of patient selection but does not affect the validity of the primary comparisons within the study cohort.

CONCLUSION

The absence of significant differences in AFS and OS across interventional treatment, deferred interventional treatment, and conservative management supports the concept that not all patients with CLTI require urgent revascularization. Outcomes appeared broadly similar among treatment strategies in selected patients; however, the study was not designed to establish equivalence between approaches. In selected patients, intervention can be reasonably delayed only when such delay is necessary or is expected to provide benefit. Conservative management should not be considered a replacement for revascularization when intervention is indicated, but may represent a reasonable management approach in carefully selected patients who are unsuitable for or do not require immediate revascularization and can yield favorable 12-month outcomes. The results of any treatment are strongly related to the patient's overall condition and comorbidities, not solely to the clinical or anatomical stage of the limb. No significant differences were observed between open and endovascular approaches in this cohort; rather, assessment should be individualized for each patient. Moreover, comorbidity burden was strongly associated with mortality in this cohort, not the treatment modality (open surgery, endovascular, etc.). Age, heart disease, COPD, previous revascularization, anemia, and moderate to severe renal failure are important predictors of poor outcomes and warrant meticulous attention.

These findings underscore the importance of individualized treatment planning and suggest that deferred intervention or conservative management may be viable alternatives in appropriately selected patients. Further prospective studies are needed to evaluate additional patient outcomes associated with delayed interventions and conservative management, to compare optimal revascularization strategies, and to assess different management approaches across subgroups defined by contemporary classification systems such as the PLAN model [1,23].

Ethics Committee Approval: The study protocol was reviewed and approved by the Institutional Ethics Committee of the Faculty of Medicine at Latakia University (Council Approval No: 1533; Approval Date: May 27, 2025).

Patient Consent for Publication: Due to the retrospective design of the study and the use of anonymized clinical data, the requirement for informed consent was waived by the committee.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: All authors contributed equally to the article.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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