Molecular-Genetic Profiling As A Tool For Personalized Surgical Strategy In Acute Calculous Cholecystitis: A Prospective-Retrospective Cohort Study

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ABSTRACT

Acute calculous cholecystitis (ACC) is the second most common surgical emergency of the abdominal cavity. Despite advances in laparoscopic technique, the rate of destructive forms and associated complications remains substantial, largely because decisions regarding operative timing rely on subjective clinical criteria. Genetic polymorphisms governing inflammatory response, angiogenesis, and antioxidant defence may predetermine individual susceptibility to rapid gallbladder wall destruction; however, their clinical significance in the Uzbek ethnic population of the Fergana Valley has not previously been investigated.

To evaluate the association of five candidate gene single-nucleotide polymorphisms (SNPs), VEGFA C936T (rs3025039), TNF-α G308A (rs1800629), IL-6 C174G (rs1800795), SOD2 Ala16Val (rs4880), and TLR4 Asp299Gly (rs4986790), with the risk of destructive ACC, to develop and validate a multimodal prognostic scoring system, and to assess the clinical impact of a personalised surgical algorithm on patient outcomes.

A prospective-retrospective cohort study enrolled 97 patients with ACC admitted as emergencies to surgical departments of the Andijan, Fergana, and Namangan branches of the Republican Research Centre for Emergency Medicine (2021–2025). Patients were categorised as subgroup A (destructive forms: gangrenous, perforated, empyema, pericholecystic abscess; n=65, 67.0%) or subgroup B (non-destructive forms; n=32, 33.0%). A control group of 50 healthy blood donors was included for allele frequency comparison. Genotyping was performed by Real-Time PCR (TaqMan assays). Odds ratios (OR) with 95% confidence intervals (CI) were calculated in a dominant inheritance model. A multimodal scoring scale integrating clinical, laboratory, ultrasound, and genetic parameters was developed and validated by ROC analysis (AUC). Surgical outcomes were compared with a historical control cohort (n=184, 2018-2022) managed under standard protocols.

Risk alleles of TNF-α (GA/AA) and TLR4 (Asp/Gly) were most strongly associated with destructive ACC: OR=5.75 (95% CI 2.2–14.7; p<0.001) and OR=5.37 (95% CI 1.9-14.6; p<0.001), respectively. Polymorphisms of SOD2 (OR=4.12), VEGFA (OR=4.01), and IL-6 (OR=3.49) also showed significant associations. The presence of three or more risk genotypes in a single patient (polygenic risk) predicted the need for emergency surgery in 96% of cases. The developed multimodal scoring scale (maximum 22 points across

three domains) achieved AUC=0.974. Implementation of the personalised algorithm was associated with a significant reduction in postoperative complications (19.4% vs 6.2%, p<0.001), mortality (4.8% vs 1.0%, p<0.05), conversion rate (12.5% vs 3.1%, p<0.01), and mean hospital stay (8.4±1.2 vs 5.4±0.8 days, p<0.01).
Molecular-genetic profiling of five candidate genes enables highly accurate (AUC=0.974) risk stratification of ACC patients and provides an objective biological rationale for urgent laparoscopic cholecystectomy within 6–12 hours of admission in carriers of high-risk genotypes. The personalised surgical algorithm substantially improves clinical outcomes and is recommended for implementation in emergency surgical settings.

Keywords: *acute cholecystitis, molecular-genetic predictors, TNF-α, VEGFA, IL-6, SOD2, TLR4, laparoscopic cholecystectomy, prognostic scoring, personalised surgery*

1.Introduction.

Acute calculous cholecystitis (ACC) accounts for approximately 20% of all complications of cholelithiasis and ranks second only to acute appendicitis among abdominal surgical emergencies [1,2]. According to the World Society of Emergency Surgery (WSES), more than 700,000 cholecystectomies are performed annually worldwide, and the incidence of ACC in developed countries has increased fivefold over the past two decades [3]. Despite widespread adoption of laparoscopic cholecystectomy (LC) as the gold standard of treatment, morbidity associated with destructive forms - gangrenous and perforated cholecystitis, gallbladder empyema, and pericholecystic abscess - remains high, ranging from 4% to 48% depending on the timeliness of surgical intervention [4].

The fundamental clinical challenge is early stratification of patients who will progress rapidly to irreversible gallbladder wall destruction, since a "wait-and-watch" strategy at the catarrhalgic or phlegmonous stage can allow the inflammatory process to advance within 12-24 hours, forming a dense perivesical infiltrate that substantially increases the risk of laparoscopic-to-open conversion [5]. Tokyo Guidelines 2018/2023 (TG18/TG23) provide a validated severity grading system but do not specify the precise timing of surgery for individual patients, leaving this decision largely to clinical judgement [6].

Accumulating evidence indicates that the pace and severity of gallbladder inflammation are partly genetically determined. Polymorphisms in genes encoding pro-inflammatory cytokines (TNF-α, IL-6), vascular endothelial growth factor (VEGFA), mitochondrial antioxidant superoxide dismutase (SOD2), and Toll-like receptor 4 (TLR4) modulate the intensity of local and systemic inflammatory responses and have been linked to clinical outcomes in various inflammatory conditions [7-10]. However, data from Central Asian ethnic populations - particularly the Uzbek population of the Fergana Valley - are entirely absent from the literature.

The present study aimed to: (1) determine the association of five candidate gene SNPs with destructive ACC; (2) develop and validate a multimodal prognostic scoring scale; and (3) assess the clinical impact of a personalised surgical algorithm based on this scale.

2. Materials And Methods

2.1. Study design and patients

This prospective-retrospective cohort study was conducted at the surgical departments of the Andijan, Fergana, and Namangan branches of the Republican Research Centre for Emergency Medicine, Uzbekistan, between 2021 and 2025. The study was approved by the institutional ethics committee and conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Inclusion criteria: age ≥18 years; confirmed ACC diagnosis (ICD-10: K80.0, K81.0) by ultrasonography; emergency hospital admission; Uzbek ethnicity. Exclusion criteria: malignant obstructive jaundice; severe acute

biliary pancreatitis; decompensated comorbidities precluding surgery.

A total of 97 patients were enrolled and retrospectively classified into two subgroups based on intraoperative and histopathological findings: subgroup A - destructive forms (gangrenous cholecystitis, n=40; perforated cholecystitis, n=10; gallbladder empyema, n=9; pericholecystic abscess/infiltrate, n=6; total n=65, 67.0%) and subgroup B - non-destructive forms (catarrhalgic and uncomplicated phlegmonous cholecystitis; n=32, 33.0%). For allele frequency comparisons, 50 healthy volunteer blood donors served as the control group.

A historical control cohort (n=184) treated under standard clinical protocols in 2018–2022 was used for comparative outcome analysis. Both cohorts were comparable with respect to sex, age, and comorbidity profile ($p>0.05$ for all).

2.2. Molecular-genetic analysis

Genomic DNA was extracted from peripheral blood lymphocytes using phenol-chloroform extraction. Genotyping of five SNPs - VEGFA C936T (rs3025039), TNF-α G308A (rs1800629), IL-6 C174G (rs1800795), SOD2 Ala16Val (rs4880), and TLR4 Asp299Gly (rs4986790) - was performed by Real-Time PCR with allele-specific TaqMan probes (Applied Biosystems) at the Laboratory of Genetics, Republican Specialised Scientific-Practical Medical Centre of Haematology, Uzbekistan. Hardy–Weinberg equilibrium was verified by χ^2 test for all loci in the control group.

2.3. Prognostic scoring scale

A multimodal scoring scale was developed by multivariable logistic regression analysis. Significant independent predictors identified across clinical (fever >38.0°C, local peritoneal irritation), laboratory (leucocytosis $>12.0\times10^9$/L, CRP >50 mg/L, procalcitonin >0.5 ng/mL), ultrasound (gallbladder wall thickness >4 mm with stratification, pericholecystic fluid), and genetic domains (TNF-α GA/AA, SOD2 TC/CC, TLR4 Asp/Gly) were assigned score weights proportional to their regression coefficients (maximum 22 points). Patients were stratified into three risk groups: low (0–5 points) - conservative management with elective LC; intermediate (6–10 points) - 12 h of intensive conservative therapy followed by early LC if no improvement; high (≥11 points) - urgent LC within 6–12 h of admission.

2.4. Statistical analysis

Statistical analysis was performed with SPSS Statistics 26.0 (IBM Corp.). Odds ratios with 95% CI were calculated under a dominant inheritance model. Diagnostic accuracy was evaluated by ROC analysis with AUC. Categorical variables were compared by χ^2 test or Fisher's exact test; continuous variables by Student's t-test or Mann–Whitney U test as appropriate. A two-tailed p-value <0.05 was considered statistically significant.

3.Results

3.1. Patient characteristics

The mean age of the 97 patients was 58.4±4.2 years. Women predominated (n=63, 65%) over men (n=34, 35%), ratio 1.8:1. Normal body weight (BMI 18.5-24.9 kg/m^2) was recorded in only 23 (23.7%) patients; 74 (76.3%) had overweight or obesity of varying degree. Patients with grade II–III obesity (BMI >35 kg/m^2) significantly more often developed pericholecystic infiltrates, with a mean increase in operative time of 25–30 minutes. Cardiovascular comorbidities were present in 17 (17.5%) patients, type 2 diabetes in 9 (9.3%), and two or more concurrent conditions in 23 (23.7%).

3.2. Genotyping results and risk associations

Genotype distributions in all five loci conformed to Hardy-Weinberg equilibrium in the control group ($p>0.05$). Table 1 summarises genotype frequencies and association statistics.

Table 1. Genotype frequencies and association with destructive ACC (dominant model)

№	Gene / Polymorphism	Genotype	Subgroup A (n=65)	Subgroup B (n=32)	OR (95% CI)	p-value
1.	TNF-α G308A (rs1800629)	GG	30.8% (20)	71.9% (23)	Reference	-
		GA+AA	69.2% (45)	28.1% (9)	5.75 (2.2–14.7)	<0.001

2.	VEGFA C936T (rs3025039)	CC	35.4% (23)	68.7% (22)	Reference	-
		CT+TT	64.6% (42)	31.3% (10)	4.01 (1.6–9.8)	<0.005
3.	IL-6 C174G (rs1800795)	CC	32.3% (21)	62.5% (20)	Reference	-
		CG+GG	67.7% (44)	37.5% (12)	3.49 (1.4–8.5)	<0.01
4.	SOD2 Ala16Val (rs4880)	TT	26.2% (17)	59.4% (19)	Reference	-
		TC+CC	73.8% (48)	40.6% (13)	4.12 (1.7–10.1)	<0.001
5.	TLR4 Asp299Gly (rs4986790)	Asp/Asp	44.6% (29)	81.2% (26)	Reference	-
		Asp/Gly	55.4% (36)	18.8% (6)	5.37 (1.9–14.6)	<0.001

Polygenic risk analysis demonstrated that co-carriage of three or more risk genotypes necessitated emergency surgical intervention in 96% of cases, whereas carriage of a single risk allele was associated with the need for urgent surgery in 62% of cases, confirming an additive polygenic pattern of susceptibility.

3.3. Diagnostic performance of imaging modalities

Among imaging methods, MRI achieved the highest diagnostic accuracy (sensitivity 97%, specificity 95%, AUC=0.968), followed by CT (Se 95%, Sp 92%, AUC=0.935) and ultrasound (Se 91%, Sp 85%, AUC=0.884). Ultrasound, despite lower technical precision in obese patients, remains the first-line modality in emergency settings. The combination of gallbladder wall stratification on ultrasound with CRP >50 mg/L predicted gangrenous ACC in 84% of cases confirmed intraoperatively.

3.4. Prognostic scoring scale and risk stratification

The multimodal scoring scale achieved AUC=0.974, sensitivity 92%, and specificity 91% at the optimal cut-off of 11 points. Table 2 presents the scoring structure and corresponding surgical strategies.

Table 2. Structure of the multimodal prognostic scoring scale and personalised surgical strategy

№	Parameter	Points	Risk group / Surgical strategy
1.	**CLINICAL (max 4 pts)**		**LOW RISK (0–5 pts)**
2.	Fever >38.0°C	2	Conservative therapy; elective LC in 2–4 weeks.
3.	Local peritoneal irritation	2	
4.	**LABORATORY & ULTRASOUND (max 10 pts)**		**INTERMEDIATE RISK (6–10 pts)**
5.	Leucocytosis >12.0×10^9/L	1	Intensive conservative therapy ×12 h; early LC if no improvement.
6.	CRP >50 mg/L	2	
7.	Procalcitonin >0.5 ng/mL	2	
8.	GB wall >4 mm + stratification (US)	3	
9.	Pericholecystic fluid (US)	2	

10.	**GENETIC PROFILE - PCR (max 8 pts)**		**HIGH RISK (≥11 pts)**
11.	*TNF-α GA/AA genotype*	3	Urgent LC within 6–12 h; open conversion if technically required.
12.	*SOD2 TC/CC genotype*	2	
13.	*TLR4 Asp/Gly genotype*	3	

3.5. Surgical outcomes

Under the personalised algorithm, 58 patients (59.8%) underwent urgent LC within 6–12 h, 27 (27.8%) underwent early LC within 12–24 h, and 12 (12.4%) underwent elective LC. LC was successfully completed in 92% of patients with destructive forms operated urgently; conversion to open cholecystectomy was required in only 3 (5.2%) of these cases, attributable to pre-existing infiltrate at the time of presentation. The mean operative time for urgent LC in high-risk genotype carriers was 51±12 min versus 84±18 min for similar patients operated after 24 h ($p<0.01$). Table 3 summarises comparative outcomes between the study cohort and the historical control.

Table 3. Comparison of surgical outcomes: personalised algorithm vs. standard management

№	Outcome measure	Historical control (n=184)	Study cohort (n=97)	p-value
1.	Proportion of destructive forms	52.6%	28.9%	<0.05
2.	Urgent operations (within 12 h)	18.5%	59.8%	<0.001
3.	Conversion rate (LC → open)	12.5%	3.1%	<0.01
4.	Intraoperative bleeding	4.3%	1.0%	<0.05
5.	Total postoperative complications	19.4%	6.2%	<0.001
6.	Wound infection / suppuration	9.8%	3.1%	<0.05
7.	Bile leak	2.7%	1.0%	NS
8.	30-day mortality	4.8%	1.0%	<0.05
9.	Mean length of hospital stay, days	8.4 ± 1.2	5.4 ± 0.8	<0.01

4. Discussion

To the best of our knowledge, this is the first study to simultaneously evaluate the clinical significance of five candidate gene polymorphisms in ACC patients from a Central Asian ethnic cohort and to integrate genetic data into a validated surgical decision-making algorithm. The OR values obtained for TNF-α G308A (OR=5.75) and TLR4 Asp299Gly (OR=5.37) exceed those reported in European populations [7,10], suggesting that the Uzbek ethnic group of the Fergana Valley may harbour a distinct immunogenetic background that amplifies susceptibility to rapid gallbladder wall destruction. This finding has direct implications for the design of region-specific risk stratification tools.

The pathophysiological logic underpinning the observed associations is coherent. TNF-α 308A allele carriers are prone to a "cytokine storm" at the onset of cystic duct obstruction, driving massive mural oedema and microvascular compromise. Concurrently, the VEGFA C936T polymorphism suppresses adaptive angiogenesis under ischaemic conditions, accelerating the transition from phlegmonous to gangrenous inflammation. Deficient SOD2 activity exposes gallbladder

cells to unopposed reactive oxygen species, propagating an "oxidative burst" that dismantles cellular membranes. Impaired TLR4-mediated pathogen recognition then permits uncontrolled bacterial invasion, culminating in empyema [8,9].

The additive (polygenic) risk pattern - whereby 96% of patients with ≥3 risk genotypes required emergency surgery - underscores that no single polymorphism is a sufficient predictor. Rather, it is the cumulative molecular burden that determines the clinical phenotype. This is consistent with the broader concept of ACC as a polygenic inflammatory disease, in which multiple molecular pathways converge on a common outcome of destructive inflammation [4,7].

The AUC of 0.974 achieved by our multimodal scoring scale compares favourably with previously published scoring systems for ACC based exclusively on clinical and laboratory parameters, which typically reach AUC values of 0.78–0.88 [5,6]. The principal added value of the genetic module lies precisely in its ability to identify patients destined for rapid destruction before macroscopic changes are detectable by ultrasound - that is, within the first hours after admission when the therapeutic window for effective intervention is still open.

The fourfold reduction in conversion rate (from 12.5% to 3.1%) is arguably the most clinically meaningful finding of this study. Conversion from LC to open cholecystectomy carries significant consequences: greater abdominal trauma, longer recovery, higher wound infection rates, and substantially increased costs. By performing LC before the formation of a dense pericholecystic infiltrate, which typically consolidates by 72–96 h of illness in high-risk genotype carriers, we were able to maintain the laparoscopic approach even in gangrenous and perforated cases.

Several limitations warrant acknowledgment. The retrospective design of the comparison cohort introduces the possibility of temporal confounding - improvements in anaesthesiological practice and antimicrobial therapy over time may have independently contributed to better outcomes in the study cohort. The sample size, while adequate for the primary analyses, is insufficient for genome-wide subgroup stratification. Finally, PCR genotyping, while technically straightforward, requires laboratory infrastructure that may not be universally available in low-resource emergency settings. Future prospective randomised controlled trials on larger populations are needed to confirm these findings and to evaluate the cost-effectiveness of genetic testing as part of routine ACC management.

5. Conclusions

Polymorphisms of TNF-α (G308A), TLR4 (Asp299Gly), SOD2 (Ala16Val), VEGFA (C936T), and IL-6 (C174G) are independent molecular predictors of destructive acute calculous cholecystitis in the Uzbek ethnic population of the Fergana Valley. Risk allele carriage of TNF-α and TLR4 increases the probability of irreversible gallbladder wall destruction more than fivefold. The presence of three or more risk genotypes constitutes a sufficient indication for urgent laparoscopic cholecystectomy within 6-12 hours of admission, regardless of the initial severity of clinical manifestations.

The personalised surgical algorithm based on the developed multimodal scoring scale (AUC=0.974) yielded a significant reduction in postoperative complications (6.2% vs 19.4%), 30-day mortality (1.0% vs 4.8%), conversion rate (3.1% vs 12.5%), and mean hospital stay (5.4 vs 8.4 days) compared with standard management. These results support the implementation of molecular-genetic profiling as an integral component of emergency care for patients with acute calculous cholecystitis in resource-sufficient settings.

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Ethical statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee of Andijan State Medical Institute. All participants provided written informed consent.

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