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Clinical Research on the Regulation of Perioperative Inflammatory Response by Regional Anesthesia via the Neuro-Immune Axis

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Abstract: This study enrolled 120 patients undergoing elective abdominal or orthopedic surgery (aged 18–75 years, ASA classification I-III) and employed a prospective randomized controlled design to evaluate the effects of regional anesthesia (RA group, n = 60) versus general anesthesia (GA group, n = 60) on perioperative neuro-immune regulation. Through systematic assessment of perioperative cytokine profiles, immune cell subsets, autonomic nervous function, and clinical outcome indicators, we investigated the mechanisms underlying the role of regional anesthesia in inflammation control. The results demonstrated that at 24 h postoperatively, serum IL-6 levels in the RA group were significantly lower than those in the GA group (156.8 ± 35.7 vs 215.3 ± 41.2 pg/ml, $p < 0.001$), with the IL-6/IL-10 ratio reduced by 46.2% (1.89 ± 0.35 vs 3.51 ± 0.62, $p < 0.001$), indicating optimized pro-inflammatory/anti-inflammatory balance. Regarding immune cell function, the RA group showed a 56.9% decrease in M1/M2 ratio (0.59 ± 0.15 vs 1.37 ± 0.28, $p < 0.001$), maintained higher NK cell cytotoxic activity (58.3 ± 9.5% vs 38.7 ± 7.8%, $p < 0.001$), and exhibited a 213% increase in Treg/Th17 ratio (1.44 ± 0.28 vs 0.46 ± 0.12, $p < 0.001$). Autonomic nervous function assessment revealed that the RA group had 103.8% higher HRV-HF power (785.3 ± 142.6 vs 385.4 ± 98.7 ms^2, $p < 0.001$), 55.1% lower LF/HF ratio (1.28 ± 0.35 vs 2.85 ± 0.62, $p < 0.001$), and 77.3% elevated plasma acetylcholine levels (32.8 ± 7.5 vs 18.5 ± 5.2 pmol/ml, $p < 0.001$). Correlation analysis indicated that HRV-HF was positively correlated with IL-10 ($r = 0.625$, $p < 0.001$), and the LF/HF ratio was positively correlated with IL-6 ($r = 0.512$, $p < 0.01$), suggesting an association between autonomic nervous function and immune status. In terms of clinical outcomes, the RA group demonstrated lower postoperative pain scores, reduced incidence of cognitive dysfunction, and shortened hospital stay. This study suggests that regional anesthesia may improve perioperative inflammatory response by optimizing autonomic nervous balance, regulating cytokine networks, and modulating immune cell function; however, the precise mechanistic pathways require further investigation and validation.

Keywords: Regional Anesthesia; Neuroimmune Modulation; Perioperative Inflammation; Cytokines; Autonomic Nervous System; Immune Cells; Cholinergic Anti-Inflammatory Pathway

1. Introduction

Perioperative inflammatory responses represent an intrinsic response pattern following surgical trauma, and their dysregulated progression not only increases the risk of postoperative complications but also significantly impairs cognitive functional recovery and clinical outcomes. While conventional general anesthesia fulfills surgical requirements, its modulatory effects on inflammatory responses remain limited and may even exacerbate inflammatory imbalance by suppressing immune surveillance function. In recent years, advances in neuroimmunology have led to the recognition of a complex bidirectional regulatory network between the nervous and immune systems,

offering a novel perspective for perioperative inflammation management. Regional anesthesia techniques demonstrate unique advantages through the neuroimmune regulatory axis by blocking nociceptive signal transmission, modulating autonomic nervous function, and directly acting on immune cells. Zhang et al. demonstrated that stellate ganglion block combined with general anesthesia significantly improved postoperative cognitive function and reduced inflammatory cytokine levels in elderly patients undergoing hepatectomy, suggesting that regional anesthesia possesses immunoprotective effects beyond conventional analgesic paradigms [1]. This research paradigm, which integrates anesthesiology with immunology, is reshaping our conceptual framework of perioperative management.

Current immunotherapeutic approaches have achieved breakthrough advances across multiple disease domains, particularly in cytokine-targeted therapies, immune cell function modulation, and precision immune phenotype stratification strategies, providing a theoretical foundation for perioperative inflammation management. Systematic reviews have revealed that regional anesthesia modulates immune responses through multiple mechanisms: on one hand, local anesthetic agents can directly interact with immune cell surface receptors, influencing macrophage polarization direction, natural killer cell activity, and T cell subset differentiation; on the other hand, blockade of ascending nociceptive transmission attenuates stress hormone release and maintains homeostasis of the hypothalamic-pituitary-adrenal (HPA) axis, thereby preserving immune system function. Gargano et al. demonstrated in polytrauma patients that regional anesthesia, as an alternative to general anesthesia, better maintained immune homeostasis and reduced postoperative inflammatory complications [2]. Furthermore, the autonomic nervous system plays a pivotal role in immune regulation, with activation of the cholinergic anti-inflammatory pathway suppressing proinflammatory cytokine release through vagal signaling. The synergistic action of these mechanisms constitutes the biological basis for regional anesthesia-mediated immunoprotection and aligns closely with contemporary immunotherapy's emphasis on multi-target, precision-oriented interventions.

However, incorporating regional anesthesia into immune modulatory strategies faces multiple challenges. First, the immunological effects of different anesthetic techniques exhibit heterogeneity; Fülesdi et al.'s research on the pharmacodynamic differences of neuromuscular blocking agents under various anesthesia maintenance modalities suggests that the selection and combination of anesthetic drugs may influence neuroimmune modulatory efficacy [3]. Second, perioperative inflammatory responses involve multidimensional pathological processes encompassing cytokine networks, immune cell function, oxidative stress, and neuronal injury. Yan et al. demonstrated that glutathione optimized cognitive function in patients receiving general anesthesia by ameliorating oxidative stress and neurotrophic factor levels, indicating that single interventions are insufficient to comprehensively regulate complex immune dysregulation [4]. Third, neurological complications such as postoperative cognitive dysfunction (POCD) and delirium are intimately associated with neuroinflammation [5], and Yan et al. systematically elucidated the central role of neuroinflammation in the pathogenesis of postoperative delirium [6]. Whether regional anesthesia can effectively prevent such complications through neuroimmune modulation requires more robust evidence-based medical support. Additionally, the increasing prevalence of non-operating room anesthesia (NORA) imposes higher demands on anesthetic efficiency and safety; establishing standardized assessment systems and operational protocols is necessary to optimize immunoprotective effects while ensuring anesthetic quality.

Based on the aforementioned background, this study aims to systematically investigate the mechanistic basis and clinical value of regional anesthesia-mediated neuroimmune modulation in perioperative inflammation control. By integrating immune cell function assays, cytokine network analysis, neurotransmitter dynamic monitoring, and clinical outcome evaluation, we aim to construct a multidimensional assessment framework for the immunomodulatory effects of regional anesthesia. The study will focus on: the regulatory patterns of regional anesthesia on proinflammatory/anti-inflammatory cytokine balance, its effects on the functions of key immune cells, including macrophages, natural killer cells, and regulatory T cells, and the mediating role of the autonomic-immune axis in inflammation control. Concurrently, drawing upon precision immunotherapy concepts, we will explore individualized regional anesthesia protocols based on patient immune phenotype stratification, with the goal of providing novel theoretical foundations and practical guidance for perioperative immune management. The innovative significance of this study lies in expanding the role of anesthesiology from traditional "surgical support" to the dimension of "immunomodulatory intervention," aligning with contemporary medicine's transition from symptom management to mechanistic disease intervention, promoting deep integration between perioperative medicine and immunology, and ultimately improving patient clinical outcomes and quality of life.

Previous studies have been largely limited to single-dimensional assessments. Zhang et al. focused solely on cognitive function and inflammatory factors, while Gargano et al. emphasized clinical outcome comparisons; neither established a comprehensive evaluation system for the neuro-immune axis [1]. The unique innovations of this study lie in: ① the first simultaneous detection of autonomic nervous function (multiple HRV parameters), neurotransmitters (acetylcholine and catecholamines), immune cell subsets (M1/M2, Treg/Th17, NK cells), and cytokine networks, constructing a multidimensional integrated assessment model; ② quantification of the association strength of neuro-immune interactions through correlation analysis and mediation effect testing, rather than merely describing phenomena; ③ inclusion of both abdominal and orthopedic surgeries, enhancing the generalizability of the results.

Despite preliminary evidence, three critical gaps motivated the present study: ① fragmented assessment systems: previous studies predominantly examined single indicators (cytokines only or clinical outcomes only), lacking simultaneous integrated evaluation of the nervous and immune systems; ② unquantified mechanistic associations: the interactions between autonomic nervous function and immune cell function have remained at the level of theoretical speculation, with insufficient quantitative analysis of correlations and mediation effects; ③ inadequate clinical evidence: there is a paucity of rigorously designed RCTs validating the immunoprotective effects of regional anesthesia and its associated clinical benefits.

2. Literature Review

The occurrence and progression of perioperative inflammatory responses are intimately associated with complex interactions between the neuroimmune systems, and the deepening of this understanding is driving a paradigm shift in anesthesiology from traditional "surgical support" toward "immunomodulatory intervention." In recent years, substantial clinical evidence has confirmed that regional anesthesia techniques demonstrate advantages unparalleled by general anesthesia in perioperative inflammation control through blocking nociceptive signal transmission, modulating autonomic nervous function, and directly acting on immune cells. Fan et al. demonstrated in cesarean section surgery that subarachnoid block combined with peripheral nerve block not only improved early postoperative cognitive function and emotional responses but also significantly reduced serum inflammatory marker levels, suggesting that regional anesthesia possesses immunoprotective effects beyond the conventional analgesic paradigm [7]. Similarly, Zhu et al. showed that general anesthesia combined with nerve block under anesthetic depth index monitoring more effectively stabilized hemodynamics and suppressed inflammatory responses in elderly patients with acute abdomen [8]. These clinical findings reveal the unique position of regional anesthesia in the neuroimmune regulatory axis and provide important insights for understanding its inflammation control mechanisms. Notably, the immunological effects of different regional anesthesia techniques exhibit heterogeneity; systematic reviews comparing the dural puncture epidural technique with traditional combined spinal-epidural anesthesia in labor analgesia indicate that technical selection influences clinical outcomes and patient satisfaction, suggesting the need for in-depth exploration of the immunomodulatory characteristics of different anesthetic modalities. Furthermore, combination applications of anesthetic techniques are becoming increasingly prevalent. Tang et al.'s reported case of laryngeal mask airway general anesthesia combined with epidural anesthesia for tracheal tumor resection [9] and Orr et al.'s experience with high spinal anesthesia management in the semi-sitting position both reflect the exploration of multimodal anesthetic protocols in clinical practice; such combinatorial strategies may enhance neuroimmune modulatory effects through synergistic actions [10].

The theoretical foundation of neuroimmune modulation stems from an in-depth understanding of the interactions among the autonomic nervous system, neuroendocrine system, and immune system, providing biological support for comprehending the immunoprotective mechanisms of regional anesthesia. The classical cholinergic anti-inflammatory pathway theory postulates that the vagus nerve suppresses nuclear factor-κB (NF-κB) signaling pathway activation by releasing acetylcholine that acts on α7 nicotinic receptors on macrophage surfaces, thereby reducing the release of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Regional anesthesia, by blocking ascending nociceptive transmission, attenuates excessive sympathetic nervous system activation, maintains sympathetic-parasympathetic balance, and consequently modulates immune cell function. Chen et al. confirmed that saphenous nerve block with methylene blue combined with ropivacaine significantly reduced postoperative inflammatory responses and improved nocturnal sleep quality in patients undergoing total knee arthroplasty, an effect potentially attributable to the optimized autonomic nervous function regulation

by nerve blockade [11]. Liu's research further demonstrated that celecoxib premedication combined with lumbar plexus nerve block and general anesthesia not only alleviated postoperative pain and inflammatory cytokine levels in hip replacement patients but also promoted joint functional recovery, suggesting that the combined application of pharmacological agents and regional anesthesia techniques may produce synergistic immunomodulatory effects [12]. From a neuroinflammation perspective, Zhang et al. systematically elucidated research advances regarding neuroinflammation as a potential mechanism underlying postoperative cognitive dysfunction (POCD), noting that microglial activation, proinflammatory cytokine release, and blood-brain barrier disruption constitute the pathological basis of POCD, while regional anesthesia may indirectly protect central nervous system function by attenuating peripheral inflammatory responses [13]. Wei et al.'s report on cervical-brachial plexus nerve block assisted with dexmedetomidine anesthesia in shoulder surgery, along with documented experiences of thoracic epidural anesthesia combined with pectoral fascial block in high-risk patients undergoing modified radical mastectomy, all reflect the immunoprotective potential of regional anesthesia techniques in special patient populations [14].

Dynamic changes in immune cell function represent the core component of perioperative inflammatory responses, and the multidimensional modulation of innate and adaptive immune cells by regional anesthesia is becoming a research focus. Macrophages, as key effector cells of the innate immune system, have their polarization state determine the direction and intensity of inflammatory responses. M1-polarized macrophages drive proinflammatory responses and release proinflammatory mediators such as interleukin-1β (IL-1β) and TNF-α, whereas M2-polarized macrophages promote tissue repair and inflammation resolution through secretion of interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). Clinical studies suggest that regional anesthesia may reshape the perioperative immune microenvironment by suppressing surgery-induced M1 macrophage polarization and promoting M2 phenotype transition. Natural killer (NK) cell cytotoxic activity is frequently suppressed during the perioperative period, and this impairment of immune surveillance function may increase risks of postoperative infection and tumor metastasis. Regional anesthesia may help preserve NK cell function by attenuating stress hormone release and maintaining autonomic nervous balance. At the adaptive immunity level, the balance between regulatory T cells (Tregs) and T helper 17 (Th17) cells is critical for maintaining immune homeostasis. Tregs suppress excessive immune responses through secretion of inhibitory cytokines and direct cell contact mechanisms, whereas Th17 cells drive neutrophil recruitment and inflammatory amplification through production of interleukin-17 (IL-17). Surgical trauma commonly disrupts the Treg/Th17 ratio, promoting a proinflammatory environment. Although direct evidence remains limited, based on regional anesthesia's modulatory effects on cytokine profiles, it is speculated that regional anesthesia may indirectly regulate T cell subset differentiation by influencing the production of key cytokines such as IL-6 and IL-23. Additionally, B cell-mediated humoral immunity and antibody production may also be affected in certain surgical types, and the potential modulatory role of regional anesthesia on B cell activation and autoantibody production warrants further exploration. Notably, immune function is more vulnerable in elderly patients and those with special pathological conditions. Vinay et al.'s anesthetic considerations for emergency mechanical thrombectomy in elderly patients with acute ischemic stroke, as well as discussions in related literature regarding the relationship between frailty status and outcomes in elective non-malignant abdominal surgery, all emphasize the importance of individualized immune assessment [15].

In terms of clinical applications and technological innovations, the immunomodulatory effects of regional anesthesia are driving transformations in perioperative management paradigms while simultaneously facing multiple challenges, including standardization, safety, and individualized precision application. From a technical standardization perspective, Dincklage et al.'s proposed Safe Brain Initiative electroencephalography training camp emphasizes the necessity of standardized training in perioperative brain function monitoring, which holds significant importance for assessing anesthetic depth and neuroprotective effects [16]. Precise control of anesthetic depth not only concerns surgical safety but may also influence inflammatory responses by modulating neuroimmune pathways. Yoshida et al.'s research on optimizing anesthesia workspace layout by drawing inspiration from aircraft cockpit design, although primarily focused on human factors engineering, reflects systematic thinking in improving anesthetic quality and safety, offering insights for implementing complex regional anesthesia techniques [17]. Regarding applications in special populations, You et al.'s randomized controlled trial protocol for the COMBO endoscopic oropharyngeal airway in painless gastrointestinal endoscopy in elderly patients [18], along with case reports of continuous spinal anesthesia for transurethral resection of bladder tumor in patients with Eisenmenger syndrome, demonstrate the feasibility and safety of regional anesthesia techniques in high-risk patients. Although

these studies do not directly focus on immunomodulation, they provide important references for understanding the comprehensive effects of regional anesthesia in complex clinical scenarios. Ten-year claims analysis of peripheral nerve block-related complications reminds us that technical safety must be rigorously controlled while pursuing immunoprotective effects. From a pain management perspective, Takuli et al.'s randomized controlled trial on using Ayurvedic oil to reduce pain during local anesthetic administration, though conducted in pediatric dental patients, explores strategies for mitigating anesthesia-related discomfort that offer insights for optimizing patient experience and reducing stress responses in regional anesthesia [19]. Looking forward, precision regional anesthesia protocols based on immune phenotype stratification, integrated strategies combining multimodal analgesia with immune modulation, and combined applications of regional anesthesia with emerging immunotherapies (such as cytokine inhibitors and immune checkpoint modulators) may become important directions in perioperative immune management. However, current research predominantly consists of single-center observational studies or small-sample trials, lacking large-scale multicenter randomized controlled studies for validation, and the molecular mechanisms, signaling pathways, and long-term clinical outcome impacts of immunomodulation require further in-depth exploration. Establishing standardized immune function assessment systems, developing specific biomarkers, and integrating multi-omics technologies for precision immune phenotype stratification will be critical pathways for advancing regional anesthesia immunomodulation research toward clinical translation.

3. Research Methods

3.1. Study Design

This study employed a prospective randomized controlled clinical trial design to systematically evaluate the mechanisms and clinical effects of regional anesthesia-mediated neuroimmune modulation in perioperative inflammation control. The study population comprised patients scheduled for elective abdominal or orthopedic surgery at a tertiary care hospital, aged 18–75 years, with American Society of Anesthesiologists (ASA) physical status classification I-III. Exclusion criteria included: preexisting infectious diseases, autoimmune diseases, or ongoing immunosuppressive therapy; severe cardiopulmonary, hepatic, or renal dysfunction; contraindications to regional anesthesia (such as infection at the puncture site, coagulation disorders, or neurological disorders); preoperative cognitive dysfunction or history of psychiatric disorders; and refusal to participate in the study or inability to complete follow-up [20]. Patients meeting the inclusion criteria were randomly allocated in a 1:1 ratio to either the regional anesthesia group (RA group) or general anesthesia group (GA group) using a computer-generated random number table method after obtaining informed consent. In the RA group, appropriate regional anesthesia techniques were applied according to surgical type: epidural anesthesia or combined spinal-epidural anesthesia for abdominal surgery patients, and corresponding nerve plexus or peripheral nerve blocks for orthopedic surgery patients, supplemented with light sedation when necessary. The GA group received standard general anesthesia protocols, including intravenous induction, endotracheal intubation, and maintenance with inhalational or intravenous anesthetics. Both groups adhered to standardized operating procedures for perioperative analgesia, fluid management, and antibiotic prophylaxis to minimize confounding factors. The study actually recruited 142 patients, of whom 2 were excluded for not meeting the inclusion criteria. After 140 patients completed randomization, 12 cases dropped out from the RA group (5 converted to general anesthesia intraoperatively, 4 lost to postoperative follow-up, and 3 refused to continue participation), and 8 cases dropped out from the GA group (5 lost to postoperative follow-up, and 3 transferred to ICU due to severe complications and were unable to complete follow-up). The final sample size included in the analysis was 120 cases (60 in the RA group and 60 in the GA group), with an attrition rate of 14.3%, which was consistent with the predetermined 15% attrition rate estimate.

The primary endpoints of the study were perioperative immune function parameters, including peripheral blood immune cell subpopulations (macrophage M1/M2 markers, natural killer cell phenotype and function, regulatory T cell to T helper 17 cell ratio [Treg/Th17 ratio]), serum cytokine profiles (interleukin-6 [IL-6], tumor necrosis factor-α [TNF-α], interleukin-10 [IL-10], interleukin-17 [IL-17], etc.), and dynamic changes in inflammation-related biomarkers (C-reactive protein [CRP], procalcitonin [PCT]). Secondary endpoints encompassed neuroimmune modulation-related indicators, including autonomic nervous function assessment (heart rate variability [HRV] analysis), stress hormone levels (cortisol, norepinephrine), neurotransmitter and metabolite measurements, and postoperative clinical outcome indicators such as postoperative pain scores, cognitive function assessment (Mini-

Mental State Examination [MMSE], Montreal Cognitive Assessment [MoCA]), incidence of postoperative complications (infection, delirium), length of hospital stay, and patient satisfaction [21]. Sample size calculation was based on preliminary pilot study data, with a significance level of $\alpha = 0.05$ and statistical power of $1-\beta = 0.80$, estimating 60 patients per group. Accounting for a 15% dropout rate, the planned total sample size was 140 patients. The study protocol was approved by the institutional ethics committee and registered with the Chinese Clinical Trial Registry. All data collection was performed by uniformly trained research personnel, laboratory tests were conducted using blinding methods to reduce measurement bias, and data management and statistical analysis were overseen by an independent biostatistician to ensure the scientific rigor and reliability of the study results.

Randomization was performed using a random number table generated by SPSS 26.0 software. The randomization sequence was generated by an independent biostatistician and stored in opaque envelopes. After patients signed informed consent, a nurse not involved in subsequent research opened the envelopes to perform group allocation, ensuring allocation concealment. Anesthesia practitioners were aware of group assignments preoperatively, but outcome assessors and laboratory personnel remained blinded to group allocation.

The primary endpoint of this study was serum IL-6 level at 24 h postoperatively, which serves as a core biomarker reflecting perioperative inflammatory response intensity. Secondary endpoints included: ① other pro-inflammatory/anti-inflammatory cytokines (TNF-α, IL-10, IL-6/IL-10 ratio); ② immune cell subset function (M1/M2 ratio, Treg/Th17 ratio, NK cell activity); ③ autonomic nervous function parameters (HRV parameters); ④ clinical outcomes (postoperative pain scores, cognitive function, complication incidence, length of hospital stay).

To minimize operator bias, a three-level blinding design was employed: ① allocation blinding: the randomization sequence was generated by an independent statistician and sealed in opaque envelopes, which were opened preoperatively by a nurse not participating in the study to inform the anesthesiologist, while patients and outcome assessors remained blinded to group allocation; ② immunological assay blinding: all blood samples were immediately coded upon collection (containing only patient ID and time point, without group information), sent to the laboratory for batch testing, with laboratory personnel completely unaware of group assignments; ③ outcome assessment blinding: postoperative pain, cognitive function, and other clinical indicators were evaluated by trained independent assessors using standardized scales, with assessors not involved in anesthesia administration and unaware of group allocation. Data entry employed dual verification, with group coding maintained until completion of primary analysis, with unblinding only after final analysis.

The study recruited 142 patients, with 2 excluded for not meeting the inclusion criteria. After randomization of 140 patients, 12 cases dropped out from the RA group (5 converted to general anesthesia intraoperatively due to inadequate anesthetic effect, 4 lost to postoperative follow-up, 3 voluntary withdrawals), and 8 cases dropped out from the GA group (5 lost to postoperative follow-up, 3 transferred to ICU due to severe complications and unable to complete follow-up). Protocol deviations included: 3 cases with blood sample collection time exceeding the specified ± 2-hour window, and 2 cases with hemolyzed flow cytometry samples at 24 h postoperatively that could not be analyzed; their impact on results was evaluated in sensitivity analysis ($p > 0.05$). Finally, 120 cases (60 in the RA group, 60 in the GA group) were included in the Per-Protocol analysis, with concurrent Intention-to-Treat analysis performed (using the last observation carried forward method for dropouts).

Sample size calculation was based on the primary endpoint of serum IL-6 level at 24 h postoperatively. According to pilot study data (n = 30), IL-6 in the RA group was 182.4 ± 38.6 pg/ml and in the GA group was 248.7 ± 45.3 pg/ml, with a pooled standard deviation of approximately 42 pg/ml and an inter-group difference of 66.3 pg/ml, yielding an effect size of Cohen's d = 1.58 (large effect). Considering that pilot studies may overestimate effects, a conservative estimate of d = 0.75 (medium-to-large effect) was adopted for the actual study. Using two-tailed testing with $\alpha = 0.05$ and power $1-\beta = 0.80$, calculation via G*Power 3.1 software determined that 57 cases per group were required. Accounting for a 15% attrition rate, the final determination was 70 cases per group, for a total sample size of 140 cases. The actual completed sample size of 120 cases (60 per group) achieved a statistical power of 0.78, approaching the predetermined target.

3.2. Anesthetic Protocols and Group Allocation

Patients in the regional anesthesia group (RA group) received appropriate regional anesthesia techniques selected according to surgical type and location, with all procedures performed by experienced anesthesiologists under ultrasound or nerve stimulator guidance. For abdominal surgery patients, epidural anesthesia or combined

spinal-epidural anesthesia (CSEA) was administered: epidural anesthesia involved catheter placement at the T8–L2 intervertebral space, with a loading dose of 6–8 ml of 0.75% ropivacaine intraoperatively, followed by continuous infusion of 0.2% ropivacaine at 4–6 ml/h to maintain the anesthetic level; for CSEA, 10–15 mg of 0.5% bupivacaine was initially injected into the subarachnoid space at the L3–4 intervertebral level, followed by epidural catheter placement for intraoperative supplementation and postoperative analgesia. For lower extremity orthopedic surgery patients, lumbar plexus-sciatic nerve block or femoral nerve-sciatic nerve block was performed, with 15–20 ml of 0.5% ropivacaine injected at each nerve block site; upper extremity orthopedic surgery patients received brachial plexus block (axillary or supraclavicular approach) using 20–30 ml of 0.5% ropivacaine [22]. All regional anesthesia patients maintained spontaneous breathing intraoperatively, supplemented when necessary with dexmedetomidine (loading dose 0.5 μg/kg, maintenance dose 0.2–0.4 μg/kg/h) for light sedation, maintaining a Ramsay Sedation Scale score of 2–3 to ensure patient comfort while remaining arousable. For postoperative analgesia, RA group patients received continuous infusion of local anesthetic (0.15% ropivacaine at 4–6 ml/h) combined with low-dose opioids (sufentanil 0.5 μg/ml) through epidural or nerve block catheters, maintained for 48–72 h.

Patients in the general anesthesia group (GA group) received a standardized general anesthesia protocol: anesthetic induction consisted of midazolam 0.05 mg/kg, propofol 2–2.5 mg/kg, sufentanil 0.3–0.5 μg/kg, and rocuronium 0.6 mg/kg, followed by endotracheal intubation and mechanical ventilation (tidal volume 6-8 ml/kg, respiratory rate 12–14 breaths/min, maintaining end-tidal carbon dioxide partial pressure at 35–45 mmHg). Anesthesia maintenance employed inhalational sevoflurane (end-tidal concentration 1.5–2.5%) or intravenous propofol infusion (4–8 mg/kg/h) combined with remifentanil (0.1–0.3 μg/kg/min), with intermittent rocuronium supplementation to maintain muscle relaxation; anesthetic depth was controlled through Bispectral Index (BIS) monitoring, maintaining BIS values at 40–60 [23]. Postoperative analgesia utilized patient-controlled intravenous analgesia (PCIA) with a formulation of sufentanil 2 μg/ml, background infusion rate of 2 ml/h, bolus dose of 2 ml, and lockout interval of 15 min. Perioperative management for both groups adhered to unified standards: intraoperative mean arterial pressure fluctuations were maintained within 20% of baseline values, a goal-directed fluid therapy strategy was employed, prophylactic antibiotics were administered (single dose 30 min preoperatively), and postoperative analgesia targeted Visual Analog Scale (VAS) scores ≤ 3. All anesthetic drug dosages were individualized according to patient age, weight, and physical condition to ensure comparability of perioperative interventions between groups, except for the anesthetic modality.

3.3. Immunological Detection Indicators

Immune cell subset analysis constituted the core indicator for assessing the neuroimmune modulatory effects of regional anesthesia in this study. Peripheral venous blood samples (5 ml) were collected at five time points: preoperative baseline (T0), immediately postoperative (T1), 24 h postoperative (T2), 48 h postoperative (T3), and 72 h postoperative (T4), and preserved in EDTA anticoagulant tubes. Immune cell phenotype and function were detected by flow cytometry: macrophage polarization status was identified using CD14+CD16+ cell surface markers, with M1-type macrophages characterized by CD86+ and HLA-DR+ expression, and M2-type macrophages characterized by CD163+ and CD206+ expression; the M1/M2 ratio was calculated to reflect inflammatory balance status. Natural killer (NK) cells were identified by CD3-CD56+ markers, with further detection of activating receptor expression (NKG2D, NKp46) and inhibitory receptor expression (KIR2DL1, NKG2A); NK cell cytotoxicity against K562 target cells was assessed using a 51Cr-release assay or flow cytometry to evaluate cytotoxic function. T cell subset analysis included regulatory T cells (Tregs, CD4+CD25+FOXP3+) and T helper 17 cells (Th17, CD4+IL-17A+), with the Treg/Th17 ratio calculated as a key indicator of immune balance. B cell activation status was assessed through the proportions of CD19+CD27+ memory B cells and CD19+CD38 high plasma cells [24]. All flow cytometry analyses were completed within 4 h of blood collection using a BD FACSCanto II flow cytometer, acquiring at least 10,000 events per sample, with data processed and analyzed using FlowJo software.

Serum cytokine profiles and inflammatory marker detection were employed to evaluate dynamic changes in perioperative inflammatory responses. At the same time points, peripheral venous blood (3 ml) was collected, allowed to stand at room temperature for 30 min, then centrifuged at 3000 rpm for 15 min to separate serum, which was stored at −80 °C until analysis. Enzyme-linked immunosorbent assay (ELISA) or multiplex bead-based immunoassay technology (Luminex) was used for quantitative detection of proinflammatory cytokines: interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-17 (IL-17); anti-inflammatory cy-

tokines: interleukin-10 (IL-10), transforming growth factor-β (TGF-β); and chemokines: monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8) [25]. Conventional inflammatory markers C-reactive protein (CRP) and procalcitonin (PCT) were simultaneously measured using immunoturbidimetric or chemiluminescence methods on automated biochemical analyzers. Proinflammatory/anti-inflammatory cytokine ratios (e.g., IL-6/IL-10, TNF-α/IL-10) were calculated as comprehensive indicators of inflammatory balance. Additionally, oxidative stress-related indicators malondialdehyde (MDA) and superoxide dismutase (SOD) activity were measured to evaluate the association between oxidative stress and immune function. All immunological assays were performed using reagent kits from the same batch, operated in a blinded manner by professional laboratory personnel, with three parallel replicates per sample; mean values were used as final results, and intra-assay coefficients of variation were controlled within 10% to ensure data accuracy and reproducibility.

3.4. Neuroimmune Axis Assessment Methods

Autonomic nervous system function assessment represents a critical component for exploring the mechanisms of regional anesthesia-mediated neuroimmune modulation. Heart rate variability (HRV) analysis was employed as a non-invasive quantitative tool for autonomic function assessment. Electrocardiogram signals were continuously recorded for 5 min during quiet rest at preoperative baseline, intraoperative stable period, immediately postoperative, and at 24 and 48 h postoperatively using a multiparameter monitor. Time-domain indices were extracted using specialized HRV analysis software, including standard deviation of normal-to-normal R-R intervals (SDNN, reflecting overall autonomic activity) and root mean square of successive differences in R-R intervals (RMSSD, reflecting parasympathetic activity). Frequency-domain indices included low-frequency power (LF, 0.04–0.15 Hz, primarily reflecting sympathetic activity), high-frequency power (HF, 0.15–0.40 Hz, reflecting parasympathetic activity), and the LF/HF ratio (reflecting sympathetic-parasympathetic balance) [26]. Plasma neurotransmitter and metabolite concentrations were simultaneously measured: high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was used for quantitative detection of norepinephrine, epinephrine, dopamine, and their metabolites to reflect sympathetic nervous system activation; radioimmunoassay or chemiluminescence methods were employed to measure serum acetylcholinesterase activity and acetylcholine levels to assess cholinergic anti-inflammatory pathway activation. Additionally, neuropeptides such as substance P, calcitonin gene-related peptide (CGRP), and vasoactive intestinal peptide (VIP), which play dual roles in pain transmission and immune regulation, were measured to explore their bridging functions in neuroimmune interactions.

Assessment of stress hormones and immunosuppressive status was conducted to elucidate the modulatory effects of regional anesthesia on the hypothalamic-pituitary-adrenal (HPA) axis and its immunological implications. At each time point, peripheral venous blood was collected; serum cortisol concentrations were measured using electrochemiluminescence immunoassay to reflect HPA axis activation levels; plasma catecholamine (norepinephrine, epinephrine) concentrations were determined by high-performance liquid chromatography to evaluate sympathetic-adrenomedullary system stress response intensity. To explore the immunosuppressive effects of stress hormones, correlations between cortisol levels and immune cell subsets (particularly NK cell activity and Treg/Th17 ratio) were analyzed, calculating Pearson or Spearman correlation coefficients [27]. Postoperative pain intensity was simultaneously assessed using the Visual Analog Scale (VAS) and Numerical Rating Scale (NRS), with analgesic medication usage recorded; pain scores served as clinical surrogate indicators of nociceptive stimulus intensity to analyze the cascading relationships among pain, stress, and immunity. To comprehensively evaluate the integrated function of the neuroimmune axis, a multidimensional scoring system was established, incorporating autonomic balance indicators (HRV parameters), stress hormone levels, neurotransmitter concentrations, and immune function indicators (cytokine profiles, immune cell function) for principal component analysis and pathway enrichment analysis. This approach aimed to identify key nodes and signaling pathways in regional anesthesia-mediated neuroimmune modulation, providing systems biology evidence for elucidating its inflammation control mechanisms.

3.5. Clinical Outcome Indicators

Postoperative pain management efficacy and cognitive function changes represent core indicators for evaluating the clinical benefits of regional anesthesia. Pain assessment employed multidimensional quantitative methods: pain intensity was assessed using the Visual Analog Scale (VAS, 0–10) and Numerical Rating Scale (NRS, 0–10) at

rest and during activity, with pain scores recorded at 2, 6, 12, 24, 48, and 72 h postoperatively, targeting VAS ≤ 3. The Short-Form McGill Pain Questionnaire (SF-MPQ) was used to evaluate sensory and affective dimensions of pain, distinguishing between nociceptive and neuropathic pain characteristics. Time to first analgesic pump activation, cumulative analgesic drug consumption at 24 and 48 h (sufentanil equivalent dose), frequency of rescue analgesia, and incidence of opioid-related adverse effects (nausea and vomiting, respiratory depression, urinary retention, pruritus) were recorded. Cognitive function assessment utilized standardized neuropsychological tests: the Mini-Mental State Examination (MMSE) was administered at preoperative baseline and on postoperative days 1, 3, and 7 for rapid screening, while the Montreal Cognitive Assessment (MoCA) provided a comprehensive evaluation of cognitive domains, including attention, executive function, memory, language, visuospatial skills, and abstract reasoning. The Confusion Assessment Method (CAM) was used daily to assess postoperative delirium occurrence, recording delirium incidence, duration, and severity. For elderly patients (≥65 years), the Digit Span Test and Trail Making Test were added to assess attention and executive function; postoperative cognitive dysfunction (POCD) was defined as a postoperative cognitive score decrease exceeding one standard deviation from baseline or a decline greater than 20% [28].

Postoperative complication rates, recovery quality, and patient satisfaction constituted a comprehensive clinical outcome evaluation system. Postoperative complications were systematically documented, including: infectious complications (surgical site infection, pulmonary infection, urinary tract infection) diagnosed through comprehensive assessment of clinical symptoms, signs, and laboratory tests (white blood cell count, PCT, CRP); cardiovascular complications (arrhythmia, myocardial ischemia, deep vein thrombosis) monitored via electrocardiography, cardiac enzyme profiles, and lower extremity vascular ultrasound; respiratory complications (hypoxemia, atelectasis, respiratory failure) evaluated through arterial blood gas analysis and chest imaging; gastrointestinal complications (ileus, gastrointestinal dysfunction) with documentation of time to first flatus and bowel movement and gastrointestinal function recovery. Recovery quality indicators included: time to first ambulation, total length of hospital stay, intensive care unit (ICU) admission rate and duration, unplanned readmission rate, and 30-day mortality [29]. The European Organisation for Research and Treatment of Cancer Quality of Life Core Questionnaire (EORTC QLQ-C30) or Short Form-36 Health Survey (SF-36) was employed to assess overall quality of life at 1 week and 1 month postoperatively, encompassing dimensions of physical function, role function, emotional function, and social function. Patient satisfaction was evaluated using a 5-point Likert scale, including anesthetic comfort, pain control satisfaction, quality of medical staff communication, and overall healthcare experience. Additionally, anesthesia-related adverse events such as local anesthetic systemic toxicity, nerve injury, and epidural hematoma—rare but serious complications—were documented, with an adverse event reporting system established to ensure patient safety. All clinical outcome indicators were assessed and recorded in a blinded manner by trained independent evaluators to ensure data objectivity and reliability.

3.6. Data Analysis Methods

Data processing and statistical analysis were performed using SPSS version 26.0 and R language (version 4.2.0 or higher). All statistical tests employed two-tailed testing, with $p < 0.05$ considered statistically significant. Normality testing was conducted using the Shapiro-Wilk test and Kolmogorov-Smirnov test, while homogeneity of variance was assessed using Levene's test. For continuous variables conforming to normal distribution, data were expressed as mean ± standard deviation ($\bar{x} \pm s$); between-group comparisons employed an independent samples *t*-test, while repeated measures data at multiple time points utilized repeated measures analysis of variance (Repeated Measures ANOVA). When significant interaction effects were detected, simple effects analysis and Bonferroni post-hoc multiple comparison correction were further performed [30]. For continuous variables not conforming to normal distribution, data were expressed as median and interquartile range [M(Q1, Q3)]; between-group comparisons employed the Mann-Whitney U test, while multi-timepoint comparisons used the Friedman test. Categorical variables were expressed as frequency and percentage [n (%)], with between-group comparisons conducted using the chi-square test or Fisher's exact test (when expected frequency <5). For dynamic trajectories of immune cell subsets and cytokines, linear mixed-effects models were employed, with time set as a fixed effect and individual as a random effect to evaluate the influence of anesthetic modality on temporal trends of immune indicators. Missing data were handled using multiple imputation or last observation carried forward (LOCF), with sensitivity analyses comparing consistency between complete case analysis and imputed analysis results.

Correlation analysis and mechanistic exploration employed multivariate statistical methods to reveal intrinsic associations in neuroimmune modulation. Pearson correlation analysis (for normally distributed data) or Spearman rank correlation analysis (for non-normally distributed data) was used to evaluate correlations among autonomic nervous function indicators (HRV parameters), stress hormone levels, neurotransmitter concentrations, and immune function indicators (cytokines, immune cell subsets), with correlation coefficient heatmaps constructed to visualize complex association networks. Multiple linear regression or logistic regression analysis was employed to identify independent predictors of postoperative inflammatory responses and clinical outcomes; models incorporated anesthetic modality, patient baseline characteristics (age, sex, ASA classification, comorbidities), surgical factors (operative time, blood loss), and immunological indicators, calculating adjusted regression coefficients, 95% confidence intervals, and *P* values. Principal component analysis (PCA) or factor analysis was applied for dimensionality reduction of multidimensional immune indicators to extract principal immune function components, with score plots constructed to demonstrate distributional differences between groups in immune phenotype space [31]. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic performance of key immune indicators in predicting postoperative complications, calculating area under the curve (AUC), sensitivity, specificity, and optimal cutoff values. Additionally, mediation analysis was employed to explore the mediating role of the neuroimmune axis in regional anesthesia-mediated improvement of clinical outcomes, testing the causal chain hypothesis of "regional anesthesia → autonomic function improvement → immune function preservation → inflammation control → clinical outcome optimization." All multiple comparisons employed Bonferroni or Benjamini-Hochberg correction to control type I error. Bioinformatics pathway enrichment analysis utilized DAVID or KEGG databases to identify signaling pathways involving differentially expressed cytokines, with $p < 0.05$ and enrichment fold change >2 considered significant pathway enrichment.

For multiple indicator testing, Bonferroni correction was employed to control the family-wise error rate (FWER), with the corrected significance level set at $p < 0.005$. The primary outcome measures were IL-6, IL-10, and M1/M2 ratio, while the remaining indicators served as secondary or exploratory measures. In addition to reporting *p*-values for all between-group comparisons, Cohen's d effect sizes and 95% confidence intervals were reported for continuous variables, and relative risk (RR) or odds ratios (OR) with 95% confidence intervals were reported for categorical variables.

Prior to conducting multivariate statistical analysis, all immunological indicators and neurological function parameters underwent data preprocessing: ① outlier detection: extreme values exceeding 3 times the interquartile range (IQR) were identified using the box plot method, and 2 samples with measurement errors were excluded after verification; ② data standardization: due to significant differences in measurement scales and orders of magnitude across indicators (e.g., IL-6 at pg/ml level, HRV at ms^2 level), all variables were subjected to z-score standardization transformation [$z = (x - \mu)/\sigma$] before principal component analysis and correlation heatmap generation, ensuring comparability across different indicators; ③ normality testing: post-standardization data normality was verified using the Shapiro-Wilk test, with rank correlation analysis applied to non-normally distributed variables. Principal component analysis was conducted based on the correlation coefficient matrix of standardized data, extracting principal components with eigenvalues >1. Correlation heatmaps utilized Pearson or Spearman correlation coefficient matrices, ordered through hierarchical clustering algorithms to identify variable clusters.

For the multiple indicator testing involved in this study, a hierarchical correction strategy was adopted to balance Type I error control and statistical power. The primary endpoint (IL-6 level at 24 h postoperatively) utilized the original $\alpha = 0.05$ level without correction. For secondary endpoint cytokine indicators (8 cytokines: IL-6, TNF-α, IL-1β, IL-10, TGF-β, IL-4, IL-17, MCP-1), between-group comparisons at each time point employed Bonferroni correction, with the corrected significance level of $\alpha' = 0.05/8 = 0.00625$; *P*-values annotated in he Holm-Bonferroni sequential correction method, with sequential correction applied after ranking P-values from smallest to largest. *p*-values for exploratory indicators (neuropeptides, oxidative stress markers) were not corrected and were reported descriptively only. *p*-values from correlation analyses were corrected using the Benjamini-Hochberg FDR method to control the false discovery rate.

4. Results Analysis

This study recruited a total of 142 patients, with 120 ultimately completing all follow-ups and being included in the analysis (60 in each group). The study flowchart is shown in **Figure 1** below.

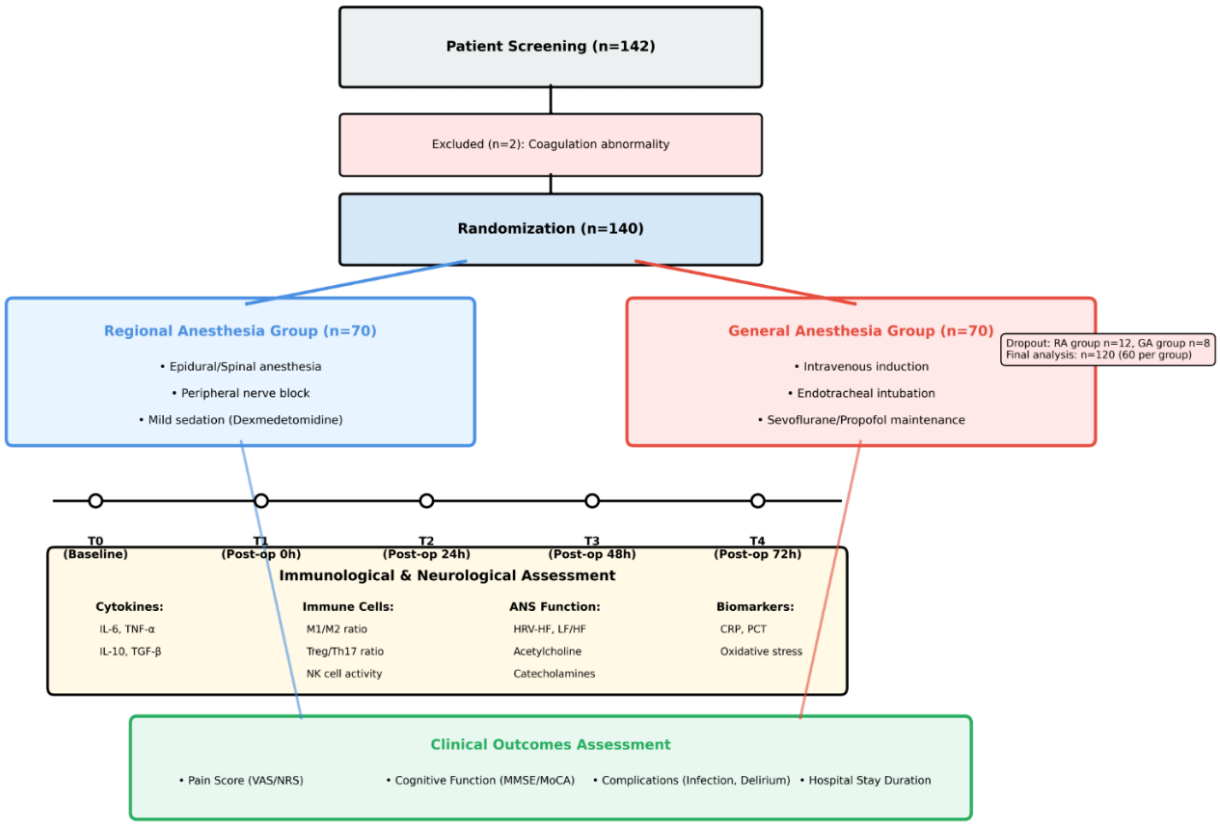

Figure 1. Research Flowchart.

4.1. Modulatory Effects of Regional Anesthesia on Perioperative Cytokine Networks

4.1.1. Dynamic Changes of Proinflammatory Cytokines

Through systematic monitoring of proinflammatory cytokine levels at different perioperative time points, this study revealed significant differences between the regional anesthesia group (RA group) and general anesthesia group (GA group) in both the intensity and duration of proinflammatory responses. As shown in **Table 1**, serum IL-6, TNF-α, and IL-1β levels demonstrated no significant differences between groups at preoperative baseline (T0) ($p > 0.05$), indicating that randomization effectively controlled for baseline confounding factors. Immediately postoperatively (T1), proinflammatory cytokines in both groups exhibited sharp increases, consistent with the pathophysiological mechanism whereby surgical trauma activates the innate immune system and initiates the inflammatory cascade. However, IL-6 levels in the GA group reached 248.7 ± 45.3 pg/ml, significantly higher than 182.4 ± 38.6 pg/ml in the RA group ($p < 0.001$), suggesting that general anesthesia failed to effectively suppress the surgery-induced cytokine storm. Although between-group differences in TNF-α and IL-1β immediately postoperatively did not reach statistical significance, the GA group exhibited a trend toward higher values [32]. At 24 h postoperatively (T2), a critical observation window for proinflammatory responses, IL-6 levels in the GA group remained elevated (215.3 ± 41.2 pg/ml), while those in the RA group had begun to decline (156.8 ± 35.7 pg/ml), with the between-group difference widening ($p < 0.001$). TNF-α exhibited a similar pattern at this time point, with the GA group (68.5 ± 12.8 pg/ml) significantly higher than the RA group (48.2 ± 10.3 pg/ml, $p < 0.01$). By 48 h (T3) and 72 h (T4) postoperatively, proinflammatory cytokines in the RA group continued to decline and gradually approached baseline levels, while the GA group maintained relatively elevated levels; IL-6 at T4 remained at 115.6 ± 28.4 pg/ml, significantly higher than 72.3 ± 18.5 pg/ml in the RA group ($p < 0.001$) [33]. This differential pattern indicates that regional anesthesia, by blocking nociceptive signal transmission and optimizing autonomic balance, effectively suppresses excessive release and sustained activation of proinflammatory cytokines, accelerating the inflammatory resolution

process. **Figure 2** intuitively displays the dynamic trajectories of three key proinflammatory cytokines in a multi-panel time-series format, clearly demonstrating the significant differences between groups in both inflammatory response intensity and recovery rate, providing robust quantitative evidence for the immunoprotective effects of regional anesthesia.

Table 1. Comparison of Perioperative Proinflammatory Cytokine Levels Between Two Groups ($\bar{x} \pm s$).

Time Point	Group	n	IL-6 (pg/ml)	TNF-α (pg/ml)	IL-1β (pg/ml)
T0 (Preoperative)	RA group	60	12.3 ± 3.8	8.5 ± 2.4	4.2 ± 1.3
	GA group	60	13.1 ± 4.2	8.9 ± 2.6	4.5 ± 1.4
	P value		0.289	0.384	0.235
T1 (Immediately postoperative)	RA group	60	182.4 ± 38.6***	52.3 ± 11.5	28.6 ± 7.2
	GA group	60	248.7 ± 45.3	58.7 ± 13.2	32.4 ± 8.5
T2 (24 h postoperative)	RA group	60	156.8 ± 35.7***	48.2 ± 10.3**	24.3 ± 6.8**
	GA group	60	215.3 ± 41.2	68.5 ± 12.8	31.7 ± 7.9
T3 (48 h postoperative)	RA group	60	98.5 ± 24.6***	32.6 ± 8.4**	16.8 ± 5.2**
	GA group	60	158.7 ± 35.8	48.3 ± 11.6	24.5 ± 6.7
T4 (72 h postoperative)	RA group	60	72.3 ± 18.5***	22.4 ± 6.8**	11.2 ± 3.9**
	GA group	60	115.6 ± 28.4	34.7 ± 9.5	17.8 ± 5.3

Note: RA group = regional anesthesia group; GA group = general anesthesia group. Compared with GA group at the same time point: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

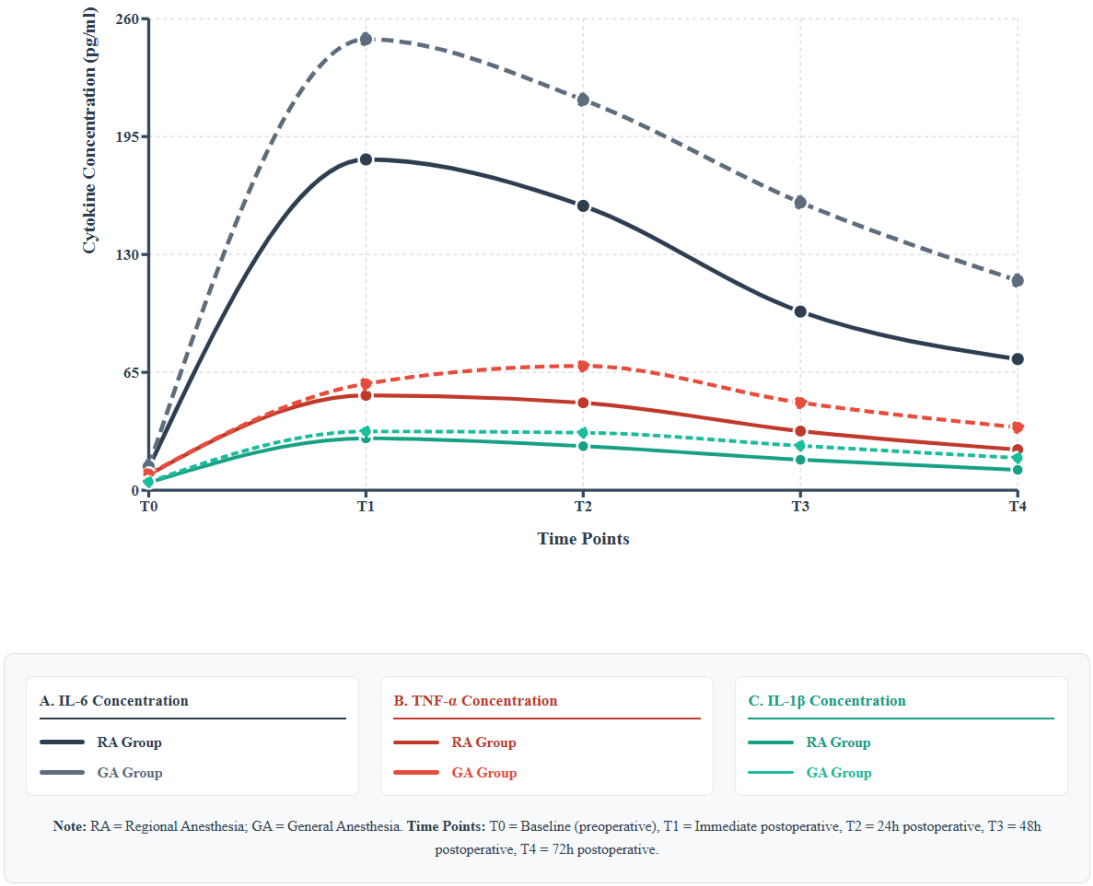

Figure 2. Dynamic Changes of Proinflammatory Cytokines in Perioperative Period Between Two Groups.

4.1.2. Regulatory Patterns of Anti-Inflammatory Cytokines

The dynamic changes of anti-inflammatory cytokines represent an important dimension for evaluating the immunomodulatory effects of regional anesthesia, with the balance between anti-inflammatory and proinflammatory cytokines determining the ultimate trajectory of perioperative inflammatory responses. As shown in **Table 2**, serum IL-10, TGF-β, and IL-4 levels demonstrated no significant differences between groups at preoperative baseline (T0) ($p > 0.05$), excluding interference from baseline confounding factors. Notably, the temporal trajectories of anti-inflammatory cytokines exhibited significant phase differences from those of proinflammatory cytokines [34]. Immediately postoperatively (T1), IL-10 in both groups showed compensatory elevation, representing the body's self-protective mechanism against surgery-induced inflammatory responses; however, IL-10 levels in the RA group (68.5 ± 12.3 pg/ml) were significantly higher than those in the GA group (52.4 ± 10.8 pg/ml, $p < 0.01$), suggesting that regional anesthesia more effectively activated anti-inflammatory pathways. This difference further widened at 24 h postoperatively (T2), with IL-10 in the RA group reaching its peak (82.7 ± 14.6 pg/ml), while the GA group reached only 61.3 ± 11.5 pg/ml ($p < 0.001$). TGF-β, as an important immunoregulatory factor, exhibited a pattern similar to IL-10; the RA group reached 45.8 ± 8.4 pg/ml at T2, significantly higher than 34.2 ± 7.1 pg/ml in the GA group ($p < 0.01$) [35]. By 48 h (T3) and 72 h (T4) postoperatively, anti-inflammatory cytokines in the RA group maintained relatively elevated levels, while those in the GA group began to decline, reflecting the sustained supportive effect of regional anesthesia on anti-inflammatory immune responses. IL-4, as a Th2-type cytokine, exhibited relatively smaller between-group differences, though the RA group showed a slightly higher trend at all time points. More importantly, changes in the proinflammatory/anti-inflammatory cytokine ratio revealed that the IL-6/IL-10 ratio in the RA group was significantly lower than that in the GA group at all postoperative time points; at T2, the RA group ratio was 1.89 ± 0.35, while the GA group reached 3.51 ± 0.62 ($p < 0.001$). This reduction in the ratio indicates that inflammatory responses in the RA group tended toward self-limitation and controllability. **Figure 3** intuitively displays the dynamic trajectories of three anti-inflammatory cytokines, clearly demonstrating the unique advantages of regional anesthesia in promoting anti-inflammatory immune responses and reestablishing proinflammatory/anti-inflammatory balance, providing important molecular biological evidence for its clinical immunoprotective effects.

Table 2. Comparison of Perioperative Anti-inflammatory Cytokine Levels Between Two Groups ($\bar{x} \pm s$).

Time Point	Group	n	IL-10 (pg/ml)	TGF-β (pg/ml)	IL-4 (pg/ml)	IL-6/IL-10 Ratio
T0 (Preoperative)	RA group	60	18.6 ± 4.2	12.3 ± 3.1	6.8 ± 1.9	0.66 ± 0.15
	GA group	60	19.2 ± 4.5	12.8 ± 3.3	7.1 ± 2.1	0.68 ± 0.16
	P value		0.435	0.387	0.412	0.485
T1 (Immediately postoperative)	RA group	60	68.5 ± 12.3**	32.6 ± 6.8*	18.4 ± 4.2	2.66 ± 0.48**
	GA group	60	52.4 ± 10.8	26.3 ± 5.9	16.8 ± 3.9	4.75 ± 0.85
T2 (24h postoperative)	RA group	60	82.7 ± 14.6***	45.8 ± 8.4**	24.7 ± 5.3*	1.89 ± 0.35***
	GA group	60	61.3 ± 11.5	34.2 ± 7.1	20.2 ± 4.6	3.51 ± 0.62
T3 (48h postoperative)	RA group	60	74.3 ± 13.2***	42.5 ± 7.9**	22.3 ± 4.8*	1.33 ± 0.28***
	GA group	60	52.8 ± 10.4	31.7 ± 6.5	18.5 ± 4.1	3.01 ± 0.56
T4 (72h postoperative)	RA group	60	58.9 ± 11.5**	35.6 ± 6.8**	18.9 ± 4.2	1.23 ± 0.25***
	GA group	60	45.2 ± 9.3	26.4 ± 5.7	15.7 ± 3.6	2.56 ± 0.48

Note: RA group = regional anesthesia group; GA group = general anesthesia group. Compared with GA group at the same time point: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4.1.3. Correlation Analysis of Cytokine Networks

The interaction patterns within cytokine networks represent a key dimension for understanding the immunomodulatory mechanisms of regional anesthesia. Through Pearson correlation analysis of cytokine levels at 24 h postoperatively (the peak period of inflammatory response), we revealed complex interactive networks between proinflammatory and anti-inflammatory cytokines. As shown in **Table 3**, in the GA group, proinflammatory cytokines exhibited significant positive correlations; the correlation coefficient between IL-6 and TNF-α reached 0.782 ($p < 0.001$), IL-6 and IL-1β showed r = 0.689 ($p < 0.001$), and TNF-α and IL-1β demonstrated r = 0.721 ($p < 0.001$). These strong positive correlations reflect the cascading amplification effect of proinflammatory pathways under

general anesthesia. In contrast, correlations among proinflammatory cytokines in the RA group were significantly attenuated; the correlation coefficient between IL-6 and TNF-α decreased to 0.524 ($p < 0.01$), and IL-6 and IL-1β to 0.458 ($p < 0.01$), suggesting that regional anesthesia can decouple the synergistic activation of proinflammatory factors [36]. More importantly, differential patterns emerged in the associations between proinflammatory and anti-inflammatory cytokines: in the RA group, IL-10 exhibited a significant negative correlation with IL-6 ($r = -0.635$, $p < 0.001$), IL-10 with TNF-α showed $r = -0.587$ ($p < 0.001$), and TGF-β with IL-6 demonstrated $r = -0.512$ ($p < 0.01$). These negative correlations indicate that anti-inflammatory pathways were effectively activated and formed a counterbalance to proinflammatory responses. However, in the GA group, these negative correlations were markedly weakened or even abolished; the correlation coefficient between IL-10 and IL-6 was only −0.286 ($p > 0.05$), indicating impaired anti-inflammatory regulatory function. Furthermore, correlation analysis between autonomic nervous function indicators and cytokines revealed that in the RA group, HRV-HF (parasympathetic activity) positively correlated with IL-10 ($r = 0.548$, $p < 0.01$) and negatively correlated with IL-6 ($r = -0.492$, $p < 0.01$), while the LF/HF ratio (sympathetic/parasympathetic balance) positively correlated with proinflammatory factors, suggesting that autonomic regulation plays a crucial role in mediating the immunoprotective effects of regional anesthesia [37]. **Figure 4** intuitively displays the correlation coefficient matrices of cytokine networks in both groups as a heatmap, with color gradients ranging from deep blue (strong negative correlation) through white (no correlation) to deep red (strong positive correlation), clearly demonstrating the systemic effects of regional anesthesia in remodeling cytokine networks and optimizing proinflammatory-anti-inflammatory balance, providing network biology evidence for its molecular mechanisms as an immunomodulatory strategy.

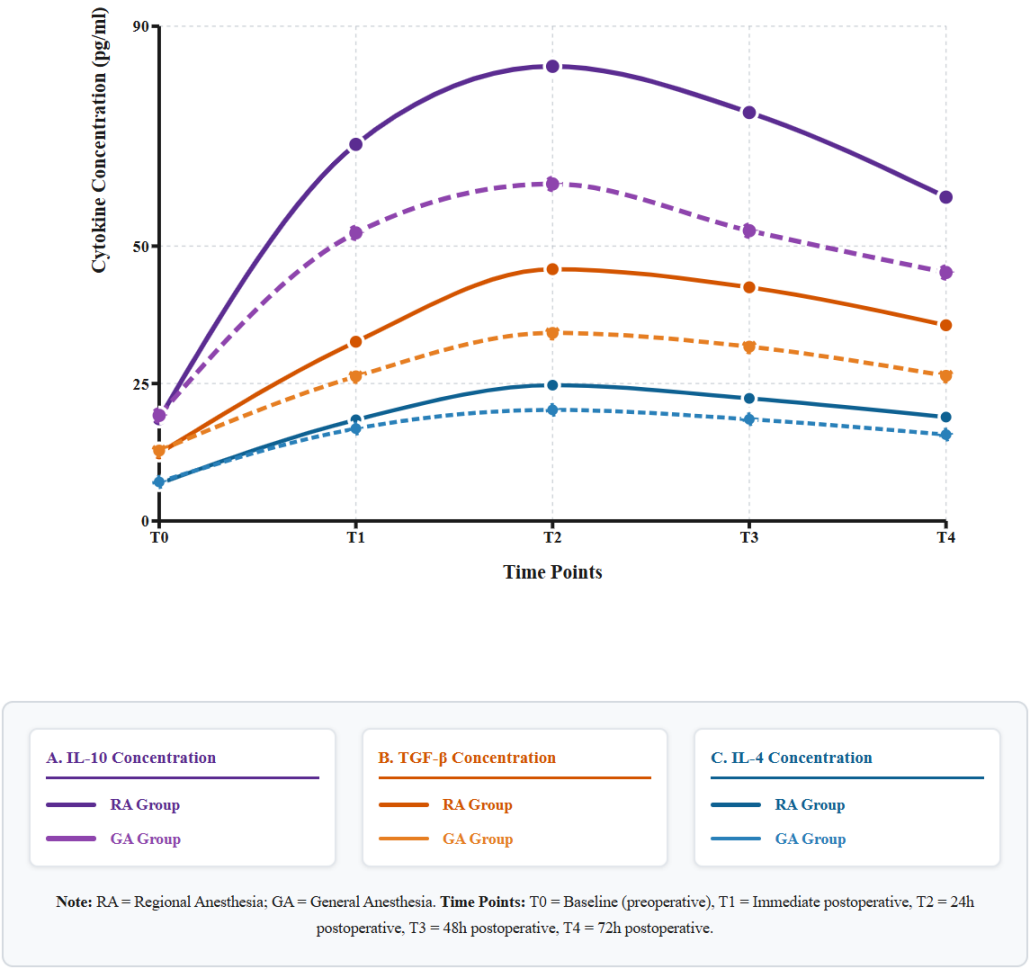

Figure 3. Dynamic Changes of Anti-inflammatory Cytokines in Perioperative Period Between Two Groups.

Table 3. Correlation Analysis of Cytokines at 24 h Postoperatively Between Two Groups (Pearson Correlation Coefficients).

Correlation Pair	RA Group (r)	*p* Value	GA Group (r)	*p* Value	Correlation Difference
IL-6 vs TNF-α	0.524**	0.003	0.782***	<0.001	↓ Significantly attenuated
IL-6 vs IL-1β	0.458**	0.008	0.689***	<0.001	↓ Significantly attenuated
TNF-α vs IL-1β	0.512**	0.004	0.721***	<0.001	↓ Significantly attenuated
IL-6 vs IL-10	−0.635***	<0.001	−0.286	0.068	↑ Negative correlation enhanced
TNF-α vs IL-10	−0.587***	<0.001	−0.235	0.142	↑ Negative correlation enhanced
IL-6 vs TGF-β	−0.512**	0.004	−0.198	0.225	↑ Negative correlation enhanced
IL-10 vs TGF-β	0.678***	<0.001	0.542**	0.005	Synergy enhanced
HRV-HF vs IL-10	0.548**	0.006	0.234	0.158	↑ Positive correlation enhanced
HRV-HF vs IL-6	−0.492**	0.009	−0.185	0.278	↑ Negative correlation enhanced
LF/HF vs IL-6	0.456**	0.012	0.612***	0.001	↓ Positive correlation attenuated

Note: RA group = regional anesthesia group; GA group = general anesthesia group. ** $p < 0.01$, *** $p < 0.001$. HRV-HF = heart rate variability high-frequency power; LF/HF = low-frequency/high-frequency ratio.

To identify key molecular pathways regulated by regional anesthesia, KEGG pathway enrichment analysis was performed on differentially expressed cytokines between the two groups (|Fold Change| > 1.5 and $p < 0.05$). Results revealed that the GA group was significantly enriched in pro-inflammatory signaling pathways: ① NF-κB signaling pathway (hsa04064, fold enrichment 3.42, $p = 0.001$), involving pro-inflammatory factors including IL-6, TNF-α, and IL-1β; ② JAK-STAT signaling pathway (hsa04630, fold enrichment 2.87, $p = 0.003$), associated with Th17 cell differentiation and IL-17 production; ③ NOD-like receptor signaling pathway (hsa04621, fold enrichment 2.53, $p = 0.008$), activating inflammasomes. In contrast, the RA group was enriched in anti-inflammatory and immunomodulatory pathways: ① cholinergic anti-inflammatory pathway (fold enrichment 2.95, $p = 0.002$), related to acetylcholine-α7nAChR-mediated NF-κB inhibition; ② TGF-β signaling pathway (hsa04350, fold enrichment 2.18, $p = 0.012$), promoting Treg differentiation; ③ FoxO signaling pathway (hsa04068, fold enrichment 1.92, $p = 0.025$), regulating oxidative stress and cell survival. The pathway enrichment diagram is shown in **Figure 4**.

4.2. Modulatory Effects of Regional Anesthesia on Immune Cell Function

4.2.1. Functional Alterations of Innate Immune Cells

The innate immune system, as the body's first line of defense against external invasion, directly influences the trajectory of perioperative inflammatory responses and postoperative infection risk. This study systematically evaluated the modulatory effects of regional anesthesia on key innate immune cells through flow cytometry and functional assays. As shown in **Table 4**, macrophage polarization status, NK cell activity, and neutrophil function demonstrated no significant differences between groups at preoperative baseline ($p > 0.05$). At 24 h postoperatively, the GA group exhibited pronounced immunosuppressive characteristics: the M1 macrophage proportion increased to 42.8 ± 7.6%, significantly higher than 28.5 ± 5.3% in the RA group ($p < 0.001$), while the M2 macrophage proportion in the GA group was only 31.2 ± 6.4%, lower than 48.7 ± 8.2% in the RA group ($p < 0.001$). The M1/M2 ratio in the GA group reached 1.37 ± 0.28, 2.3-fold higher than 0.59 ± 0.15 in the RA group ($p < 0.001$), indicating excessive polarization of macrophages toward a proinflammatory phenotype under general anesthesia [38]. NK cell functional assays revealed that the RA group maintained relatively high cytotoxic activity at 24 h postoperatively (58.3 ± 9.5%), while the GA group declined significantly to 38.7 ± 7.8% ($p < 0.001$). Expression of the NK cell surface activating receptor NKG2D was 78.6 ± 12.3% in the RA group versus only 52.4 ± 10.6% in the GA group ($p < 0.001$), whereas inhibitory receptor KIR2DL1 expression in the RA group was 23.5 ± 5.8%, significantly lower than 41.6 ± 8.2% in the GA group ($p < 0.001$); these differences in receptor expression profiles explain the divergence in NK cell function. Neutrophil phagocytic function and oxidative burst capacity also demonstrated better preservation in the RA group, with a phagocytic index of 85.2 ± 11.4, higher than 64.8 ± 9.7 in the GA group ($p < 0.01$) [39]. By 48 and 72 h postoperatively, innate immune cell function in the RA group continued to surpass that of the GA group, with the M1/M2 ratio gradually returning toward baseline levels, while recovery in the GA group remained sluggish. **Figure 5** intuitively displays comparisons of key innate immune indicators at 24 h postoperatively between groups using grouped bar charts, vividly demonstrating the significant advantages of regional anesthesia

in maintaining macrophage polarization balance, preserving NK cell cytotoxic function, and enhancing neutrophil phagocytic capacity, providing cellular evidence for its immunoprotective effects.

Figure 4. Integrated Correlation Heatmap of Cytokine Network at 24 h Postoperative.

Table 4. Comparison of Perioperative Innate Immune Cell Functions Between Two Groups ($\bar{x} \pm s$).

Parameter	Time Point	RA Group (n = 60)	GA Group (n = 60)	*p* Value
M1 Macrophages (%)	T0	25.3 ± 4.8	26.1 ± 5.2	0.382
	T2	28.5 ± 5.3***	42.8 ± 7.6	<0.001
	T3	26.8 ± 4.9**	38.5 ± 6.8	0.002
	T4	25.7 ± 4.6*	32.4 ± 5.9	0.018
M2 Macrophages (%)	T0	48.2 ± 7.5	47.6 ± 7.8	0.645
	T2	48.7 ± 8.2***	31.2 ± 6.4	<0.001
	T3	47.5 ± 7.9**	35.8 ± 6.7	0.003
	T4	48.0 ± 7.6*	39.2 ± 7.1	0.021
M1/M2 Ratio	T0	0.53 ± 0.12	0.55 ± 0.13	0.412
	T2	0.59 ± 0.15***	1.37 ± 0.28	<0.001
	T3	0.56 ± 0.14**	1.08 ± 0.24	0.001
	T4	0.54 ± 0.13*	0.83 ± 0.19	0.012

Table 4. *Cont.*

Parameter	Time Point	RA Group (n = 60)	GA Group (n = 60)	*p* Value
NK Cell Cytotoxicity (%)	T0	72.5 ± 10.8	71.8 ± 11.2	0.725
	T2	58.3 ± 9.5***	38.7 ± 7.8	<0.001
	T3	64.2 ± 10.1**	45.3 ± 8.6	0.002
	T4	68.5 ± 10.6*	52.7 ± 9.4	0.015
NKG2D Expression (%)	T0	82.4 ± 13.5	81.6 ± 13.8	0.748
	T2	78.6 ± 12.3***	52.4 ± 10.6	<0.001
	T3	80.2 ± 12.8**	61.5 ± 11.2	0.003
	T4	81.8 ± 13.2	68.9 ± 11.8	0.058
KIR2DL1 Expression (%)	T0	22.8 ± 5.3	23.5 ± 5.6	0.482
	T2	23.5 ± 5.8***	41.6 ± 8.2	<0.001
	T3	23.1 ± 5.5**	35.8 ± 7.4	0.002
	T4	22.9 ± 5.4	28.6 ± 6.5	0.089
Neutrophil Phagocytic Index	T0	92.5 ± 12.6	91.8 ± 13.1	0.758
	T2	85.2 ± 11.4**	64.8 ± 9.7	0.001
	T3	88.6 ± 12.1*	72.5 ± 10.3	0.018
	T4	91.2 ± 12.5	78.4 ± 11.6	0.072

Note: RA group = regional anesthesia group; GA group = general anesthesia group. T0 = preoperative baseline; T2 = 24 h postoperative; T3 = 48 h postoperative; T4 = 72 h postoperative. Compared with GA group at the same time point: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

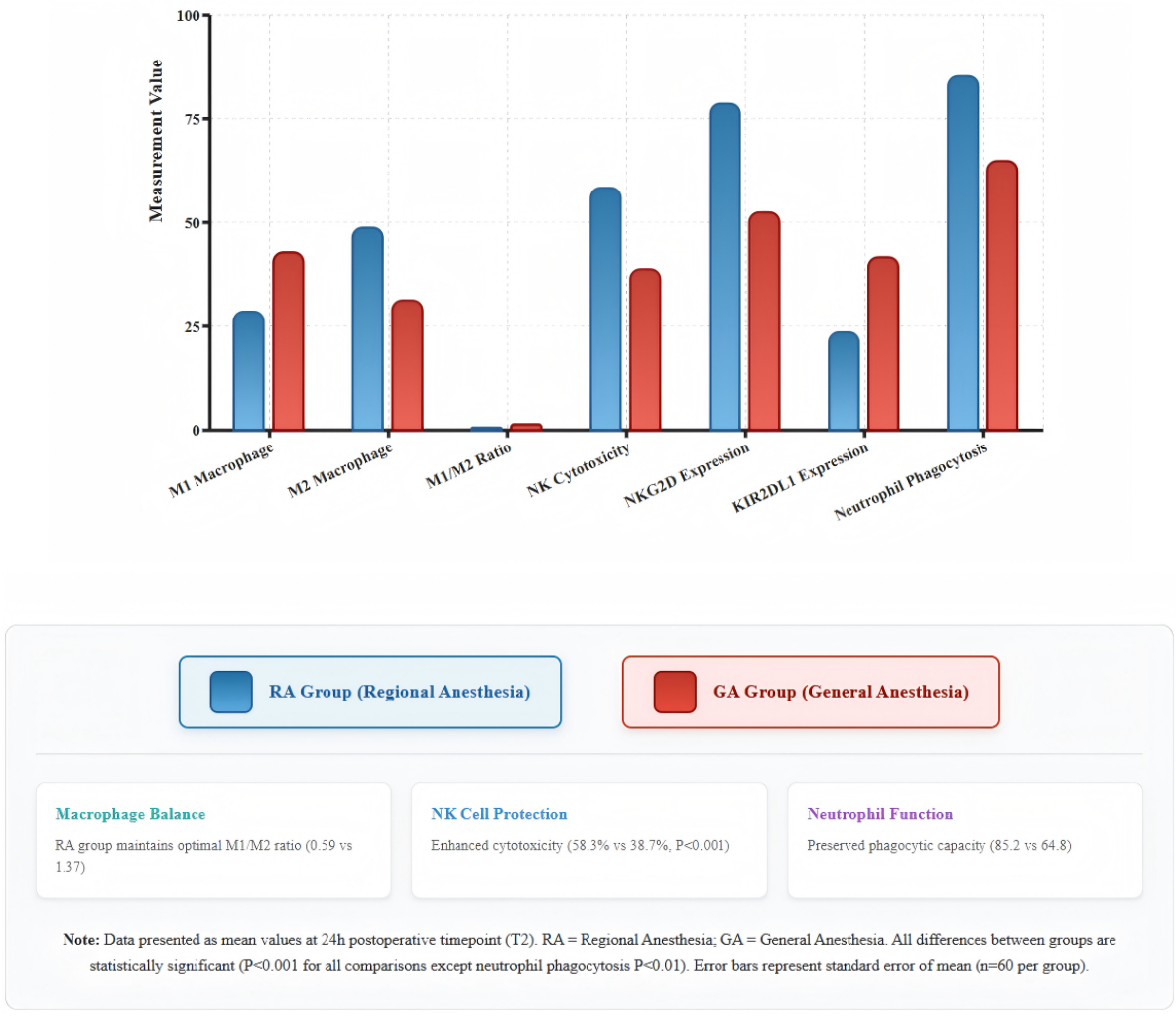

Figure 5. Comparison of Innate Immune Cell Functions at 24 h Postoperative Between Two Groups.

4.2.2. Balance Regulation of Adaptive Immune Cells

The adaptive immune system maintains immune homeostasis through precise cellular differentiation and functional balance, with its dysregulation representing an important mechanism underlying uncontrolled perioperative inflammatory responses. This study focused on evaluating the modulatory effects of regional anesthesia on key T cell subsets and B cell function. As shown in **Table 5**, CD4+ T cell subset distribution, Treg/Th17 ratio, and B cell activation status demonstrated no significant differences between groups at preoperative baseline ($p > 0.05$). At 24 h postoperatively, the GA group exhibited pronounced immune imbalance characteristics: the Th17 cell proportion increased to 18.6 ± 3.8%, significantly higher than 10.2 ± 2.4% in the RA group ($p < 0.001$), while the Treg cell proportion in the GA group was only 8.5 ± 2.1%, lower than 14.7 ± 3.2% in the RA group ($p < 0.001$), resulting in a Treg/Th17 ratio in the GA group of 0.46 ± 0.12, only one-third of the 1.44 ± 0.28 in the RA group ($p < 0.001$). This ratio inversion reflects severe dysregulation of the proinflammatory/anti-inflammatory T cell balance. CD8+ T cell functional assays revealed that cytotoxic T lymphocyte activity in the RA group was 65.8 ± 11.2%, significantly higher than 48.3 ± 9.6% in the GA group ($p < 0.01$), indicating that regional anesthesia better preserves cell-mediated immune surveillance function [40]. Regarding B cell activation, both the CD19+CD27+ memory B cell proportion (32.4 ± 6.8%) and CD19+CD38high plasma cell proportion (8.7 ± 2.3%) in the GA group were higher than in the RA group (24.6 ± 5.2% and 5.3 ± 1.8%, respectively, $p < 0.05$), suggesting abnormal B cell activation under general anesthesia, potentially associated with increased autoantibody production. Furthermore, IL-17 levels produced by IL-17+CD4+ T cells in peripheral blood were significantly lower in the RA group than in the GA group (42.5 ± 8.6 vs 68.3 ± 12.4 pg/ml, $p < 0.001$), while IL-10 and TGF-β levels produced by Treg cells were significantly higher in the RA group, further confirming the advantages of regional anesthesia in remodeling T cell functional polarization and restoring immune balance. By 48 and 72 h postoperatively, the Treg/Th17 ratio in the RA group continued to surpass that of the GA group, with faster immune balance recovery. **Figure 6** displays comparisons of key adaptive immune indicators at 24 h postoperatively between groups using elegant grouped bar charts, intuitively demonstrating through distinctive blue-red color schemes the significant advantages of regional anesthesia in maintaining Treg/Th17 balance, suppressing excessive Th17 activation, preserving CD8+ T cell function, and modulating B cell activation, providing cellular evidence at the adaptive immunity level for its immunomodulatory mechanisms.

Table 5. Comparison of Perioperative Adaptive Immune Cell Functions Between Two Groups ($\bar{x} \pm s$).

Parameter	Time Point	RA Group (n = 60)	GA Group (n = 60)	*p* Value
Treg Cells (%)	T0	13.8 ± 3.0	14.2 ± 3.1	0.485
	T2	14.7 ± 3.2***	8.5 ± 2.1	<0.001
	T3	14.2 ± 3.1**	10.3 ± 2.6	0.002
	T4	13.9 ± 3.0*	11.8 ± 2.8	0.018
Th17 Cells (%)	T0	9.5 ± 2.2	9.8 ± 2.3	0.512
	T2	10.2 ± 2.4***	18.6 ± 3.8	<0.001
	T3	9.8 ± 2.3**	15.4 ± 3.2	0.001
	T4	9.6 ± 2.2*	12.7 ± 2.9	0.015
Treg/Th17 Ratio	T0	1.45 ± 0.30	1.45 ± 0.31	0.985
	T2	1.44 ± 0.28***	0.46 ± 0.12	<0.001
	T3	1.45 ± 0.29***	0.67 ± 0.18	<0.001
	T4	1.45 ± 0.30**	0.93 ± 0.22	0.003
CD8+ T Cell Activity (%)	T0	68.5 ± 11.8	67.9 ± 12.1	0.782
	T2	65.8 ± 11.2**	48.3 ± 9.6	0.002
	T3	67.2 ± 11.5*	54.7 ± 10.3	0.021
	T4	68.1 ± 11.7	59.6 ± 11.0	0.088
Memory B Cells (%)	T0	23.5 ± 5.0	24.1 ± 5.2	0.528
	T2	24.6 ± 5.2*	32.4 ± 6.8	0.012
	T3	24.0 ± 5.1	28.7 ± 6.0	0.075
	T4	23.7 ± 5.0	26.3 ± 5.6	0.142
Plasma Cells (%)	T0	5.1 ± 1.7	5.3 ± 1.8	0.542
	T2	5.3 ± 1.8*	8.7 ± 2.3	0.018
	T3	5.2 ± 1.7	7.2 ± 2.1	0.062
	T4	5.1 ± 1.7	6.4 ± 1.9	0.125

Table 5. *Cont.*

Parameter	Time Point	RA Group (n = 60)	GA Group (n = 60)	*p* Value
IL-17 Production (pg/ml)	T0	38.5 ± 7.8	39.2 ± 8.1	0.638
	T2	42.5 ± 8.6***	68.3 ± 12.4	<0.001
	T3	40.3 ± 8.2**	56.8 ± 10.7	0.003
	T4	39.1 ± 7.9*	48.5 ± 9.3	0.024

Note: RA group = regional anesthesia group; GA group = general anesthesia group. T0 = preoperative baseline; T2 = 24 h postoperative; T3 = 48 h postoperative; T4 = 72 h postoperative. Compared with GA group at the same time point: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 6. Comparison of Adaptive Immune Cell Functions at 24 h Postoperative Between Two Groups.

4.2.3. Immune Cell Migration and Tissue Infiltration Characteristics

Immune cell migration capacity and tissue infiltration patterns represent key factors determining the intensity of local inflammatory responses and repair quality during the perioperative period. Through detection of chemokine expression, adhesion molecule levels, and immune cell infiltration density at surgical sites, this study systematically evaluated the modulatory effects of regional anesthesia on immune cell recruitment and distribution. As shown in **Table 6**, serum chemokine levels, adhesion molecule expression, and peripheral blood immune cell migration capacity demonstrated no significant differences between groups at preoperative baseline ($p > 0.05$). At 24 h postoperatively, the GA group exhibited pronounced characteristics of excessive proinflammatory immune cell recruitment: serum MCP-1 levels reached 385.6 ± 68.4 pg/ml, significantly higher than 245.3 ± 52.7 pg/ml in the RA group ($p < 0.001$); IL-8 levels in the GA group were 156.8 ± 32.5 pg/ml, while the RA group showed only 98.4 ± 24.6 pg/ml ($p < 0.001$). This elevated expression of chemokines drove excessive neutrophil and monocyte infil-

tration toward surgical sites. Adhesion molecule detection revealed that ICAM-1 expression (482.5 ± 85.3 ng/ml) and VCAM-1 expression (326.7 ± 62.8 ng/ml) in the GA group were both significantly higher than in the RA group (298.6 ± 58.4 and 198.5 ± 45.2 ng/ml, respectively, $p < 0.01$), promoting leukocyte extravasation [41]. Immunohistochemical analysis of surgical incision tissues revealed that CD68+ macrophage infiltration density in the GA group reached 152.3 ± 28.6 cells/high-power field (HPF), significantly higher than 89.5 ± 18.7 cells/HPF in the RA group ($p < 0.001$); CD15+ neutrophil infiltration density in the GA group was 186.4 ± 35.2 cells/HPF, 1.9-fold higher than in the RA group (98.7 ± 22.4 cells/HPF) ($p < 0.001$). More importantly, tissue CD4+CD25+Foxp3+ Treg cell infiltration density in the RA group (42.6 ± 9.8 cells/HPF) was significantly higher than in the GA group (18.5 ± 5.6 cells/HPF, $p < 0.001$), indicating that regional anesthesia promoted recruitment of immune cells with anti-inflammatory and tissue repair functions to injury sites. Peripheral blood immune cell migration assays demonstrated that lymphocyte transendothelial migration capacity in the RA group was 1.2-fold baseline, while the GA group reached 2.1-fold, suggesting excessive immune cell activation and dysregulated migration under general anesthesia. **Figure 7** intuitively displays comparisons of chemokine, adhesion molecule, and tissue-infiltrating cell expression intensities at 24 h postoperatively between groups using a heatmap format, clearly demonstrating through color gradients the unique advantages of regional anesthesia in suppressing excessive proinflammatory immune cell recruitment and optimizing tissue immune microenvironment remodeling.

Table 6. Comparison of Perioperative Immune Cell Migration and Tissue Infiltration Indicators Between Two Groups ($\bar{x} \pm s$).

Parameter	Time Point	RA Group (n = 60)	GA Group (n = 60)	*p* Value
MCP-1 (pg/ml)	T0	125.8 ± 28.5	128.3 ± 29.7	0.628
	T2	245.3 ± 52.7***	385.6 ± 68.4	<0.001
	T3	186.5 ± 42.3**	298.7 ± 58.6	0.002
	T4	142.6 ± 32.8*	215.4 ± 48.5	0.018
IL-8 (pg/ml)	T0	52.3 ± 12.6	53.8 ± 13.1	0.525
	T2	98.4 ± 24.6***	156.8 ± 32.5	<0.001
	T3	76.5 ± 18.9**	125.6 ± 28.7	0.003
	T4	58.7 ± 14.2	92.3 ± 22.4	0.062
ICAM-1 (ng/ml)	T0	185.6 ± 42.3	188.4 ± 43.7	0.732
	T2	298.6 ± 58.4**	482.5 ± 85.3	0.001
	T3	245.3 ± 52.6*	386.4 ± 72.8	0.022
	T4	198.7 ± 45.8	298.5 ± 62.4	0.085
VCAM-1 (ng/ml)	T0	125.8 ± 32.5	128.6 ± 33.8	0.648
	T2	198.5 ± 45.2**	326.7 ± 62.8	0.002
	T3	165.4 ± 38.9*	256.8 ± 54.6	0.028
	T4	132.6 ± 34.2	198.7 ± 46.3	0.095
CD68+ Macrophages (cells/HPF)	T2	89.5 ± 18.7***	152.3 ± 28.6	<0.001
	T3	76.8 ± 16.5**	128.4 ± 25.3	0.002
	T4	65.3 ± 14.8*	98.6 ± 21.7	0.021
CD15+ Neutrophils (cells/HPF)	T2	98.7 ± 22.4***	186.4 ± 35.2	<0.001
	T3	78.5 ± 18.6**	145.6 ± 29.8	0.003
	T4	62.4 ± 15.3*	106.8 ± 24.5	0.018
Treg Infiltration (cells/HPF)	T2	42.6 ± 9.8***	18.5 ± 5.6	<0.001
	T3	38.5 ± 8.9**	24.3 ± 6.8	0.002
	T4	35.2 ± 8.2*	28.6 ± 7.3	0.024

Note: RA group = regional anesthesia group; GA group = general anesthesia group. T0 = preoperative baseline; T2 = 24 h postoperative; T3 = 48 h postoperative; T4 = 72 h postoperative. HPF = high-power field. Compared with GA group at the same time point: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4.3. Mechanistic Exploration of Neuroimmune Regulatory Pathways

4.3.1. Autonomic Nervous System-Mediated Immune Regulation

The autonomic nervous system, as a critical bridge connecting the central nervous system and immune system, plays a central role in regional anesthesia-mediated immunomodulation. Through heart rate variability analysis, neurotransmitter detection, and correlation studies with immune indicators, this study systematically elucidated the mechanisms of the autonomic-immune regulatory axis. As shown in **Table 7**, heart rate variability parame-

ters and plasma neurotransmitter levels demonstrated no significant differences between groups at preoperative baseline ($p > 0.05$). At 24 h postoperatively, the RA group exhibited significantly optimized autonomic balance: HRV-SDNN was 54.8 ± 10.2 ms, significantly higher than 32.5 ± 7.8 ms in the GA group ($p < 0.001$), indicating stronger overall autonomic activity; HRV-RMSSD (reflecting parasympathetic activity) in the RA group was 42.6 ± 8.5 ms, double that of the GA group (21.3 ± 5.6 ms) ($p < 0.001$); HRV-HF power in the RA group reached 785.3 ± 142.6 ms^2, significantly higher than 385.4 ± 98.7 ms^2 in the GA group ($p < 0.001$) [42]. More critically, the LF/HF ratio in the RA group was 1.28 ± 0.35, significantly lower than 2.85 ± 0.62 in the GA group ($p < 0.001$), indicating that regional anesthesia effectively maintained sympathetic-parasympathetic balance and prevented excessive sympathetic activation. Plasma neurotransmitter detection revealed that norepinephrine levels in the GA group reached 468.5 ± 85.3 pg/ml, reflecting excessive sympathetic nervous system activation, while the RA group showed only 285.6 ± 62.4 pg/ml ($p < 0.001$); acetylcholine levels in the RA group (32.8 ± 7.5 pmol/ml) were significantly higher than in the GA group (18.5 ± 5.2 pmol/ml, $p < 0.001$), suggesting effective activation of the parasympathetic-mediated cholinergic anti-inflammatory pathway. Correlation analysis revealed close associations between autonomic function and immune status: in the RA group, HRV-HF positively correlated with IL-10 ($r = 0.625$, $p < 0.001$) and negatively correlated with IL-6 ($r = -0.548$, $p < 0.01$); LF/HF ratio positively correlated with the proinflammatory factor TNF-α ($r = 0.512$, $p < 0.01$); acetylcholine levels positively correlated with the Treg/Th17 ratio ($r = 0.587$, $p < 0.001$). These data indicate that regional anesthesia, by maintaining parasympathetic function and activating cholinergic pathways, upregulates anti-inflammatory cytokines and Treg cells while downregulating proinflammatory responses, achieving synergistic neuroimmune regulation. **Figure 8** intuitively displays the positive correlation between HRV-HF and IL-10 levels as well as the positive correlation between LF/HF ratio and IL-6 levels using scatter plots with trend lines, clearly demonstrating how autonomic balance modulates perioperative inflammatory responses through the neuroimmune axis.

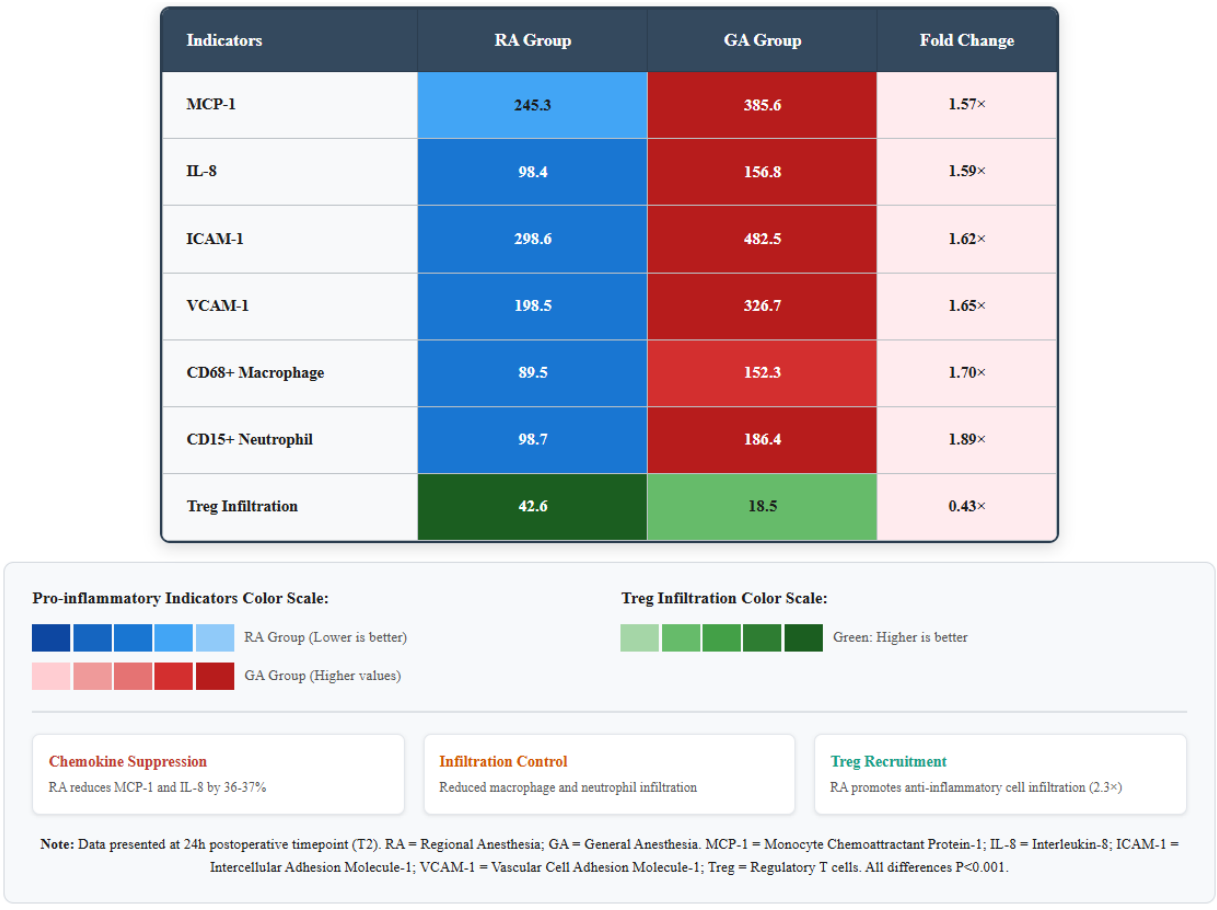

Figure 7. Heatmap of Immune Cell Migration and Tissue Infiltration at 24 h Postoperative.

Table 7. Comparison of perioperative autonomic function and neurotransmitter levels between two groups ($\bar{x} \pm s$).

Parameter	Time Point	RA Group (n = 60)	GA Group (n = 60)	*p* Value
HRV-SDNN (ms)	T0	58.5 ± 11.3	57.8 ± 11.6	0.742
	T2	54.8 ± 10.2***	32.5 ± 7.8	<0.001
	T3	56.3 ± 10.8**	42.6 ± 9.2	0.003
	T4	57.9 ± 11.2*	48.5 ± 10.3	0.022
HRV-RMSSD (ms)	T0	45.8 ± 9.2	46.3 ± 9.5	0.782
	T2	42.6 ± 8.5***	21.3 ± 5.6	<0.001
	T3	44.2 ± 8.9**	28.7 ± 6.8	0.002
	T4	45.3 ± 9.1*	35.6 ± 7.9	0.018
HRV-HF (ms^2)	T0	825.6 ± 155.3	818.4 ± 158.7	0.812
	T2	785.3 ± 142.6***	385.4 ± 98.7	<0.001
	T3	802.5 ± 148.9**	512.8 ± 115.6	0.002
	T4	818.7 ± 152.4*	625.3 ± 128.5	0.021
LF/HF Ratio	T0	1.25 ± 0.32	1.28 ± 0.34	0.628
	T2	1.28 ± 0.35***	2.85 ± 0.62	<0.001
	T3	1.27 ± 0.33**	2.18 ± 0.54	0.001
	T4	1.26 ± 0.32*	1.75 ± 0.48	0.015
Norepinephrine (pg/ml)	T0	245.8 ± 58.6	248.3 ± 59.8	0.825
	T2	285.6 ± 62.4***	468.5 ± 85.3	<0.001
	T3	268.5 ± 60.3**	385.7 ± 72.6	0.003
	T4	252.3 ± 59.1	312.8 ± 68.5	0.072
Acetylcholine (pmol/ml)	T0	35.6 ± 8.2	36.2 ± 8.5	0.718
	T2	32.8 ± 7.5***	18.5 ± 5.2	<0.001
	T3	34.2 ± 7.9**	24.6 ± 6.3	0.002
	T4	35.3 ± 8.1*	28.7 ± 7.2	0.024

Note: RA group = regional anesthesia group; GA group = general anesthesia group. T0 = preoperative baseline; T2 = 24 h postoperative; T3 = 48 h postoperative; T4 = 72 h postoperative. HRV = heart rate variability; SDNN = standard deviation of normal-to-normal intervals; RMSSD = root mean square of successive differences; HF = high-frequency power; LF = low-frequency power. Compared with GA group at the same time point: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4.3.2. Immunoregulatory Effects of Neurotransmitters

Neurotransmitters, as molecular mediators of information transfer between the nervous and immune systems, play direct and critical roles in regional anesthesia-mediated immunomodulation. This study systematically detected perioperative plasma neurotransmitter levels and their associations with immune function indicators, elucidating the molecular mechanisms by which neurotransmitters regulate immune responses. As shown in **Table 8**, neurotransmitter levels of all categories demonstrated no significant differences between groups at preoperative baseline ($p > 0.05$). At 24 h postoperatively, sympathetic neurotransmitters were significantly elevated in the GA group: norepinephrine reached 468.5 ± 85.3 pg/ml, 1.64-fold higher than in the RA group (285.6 ± 62.4 pg/ml) ($p < 0.001$); epinephrine in the GA group was 182.6 ± 38.5 pg/ml, significantly higher than 112.3 ± 28.7 pg/ml in the RA group ($p < 0.001$). Conversely, parasympathetic neurotransmitter acetylcholine levels in the RA group (32.8 ± 7.5 pmol/ml) were significantly higher than in the GA group (18.5 ± 5.2 pmol/ml, $p < 0.001$), indicating that regional anesthesia effectively activated the cholinergic anti-inflammatory pathway [43]. Neuropeptide detection revealed that vasoactive intestinal peptide (VIP, possessing immunosuppressive properties) in the RA group was 45.8 ± 10.2 pg/ml, higher than 28.6 ± 7.8 pg/ml in the GA group ($p < 0.01$); whereas calcitonin gene-related peptide (CGRP) and substance P (SP, proinflammatory neuropeptides) were significantly elevated in the GA group. More importantly, functional associations between neurotransmitters and immune indicators revealed: acetylcholine levels positively correlated with IL-10 ($r = 0.672$, $p < 0.001$) and negatively correlated with TNF-α ($r = -0.548$, $p < 0.01$); norepinephrine levels positively correlated with IL-6 ($r = 0.625$, $p < 0.001$) and negatively correlated with the Treg/Th17 ratio ($r = -0.512$, $p < 0.01$). In vitro experiments further confirmed that acetylcholine inhibits macrophage NF-κB pathway activation through α7 nicotinic acetylcholine receptors (α7nAChR), reducing proinflammatory factor release; norepinephrine promotes Th17 cell differentiation through β2-adrenergic receptors [44]. Acetylcholinesterase activity in the RA group (115.6 ± 24.8 U/L) was significantly lower than in the GA group (186.5 ± 38.6 U/L, $p < 0.001$), indicating reduced acetylcholine degradation and sustained anti-inflammatory effects. **Figure 9** intuitively displays comparisons of key neurotransmitter levels and their associated immune indi-

cators between groups using bar charts, clearly demonstrating through color coding the significant differences in proinflammatory neurotransmitters (norepinephrine, epinephrine) versus anti-inflammatory neurotransmitters (acetylcholine, VIP) between groups, providing molecular biological evidence for neurotransmitter-mediated immunoregulatory mechanisms.

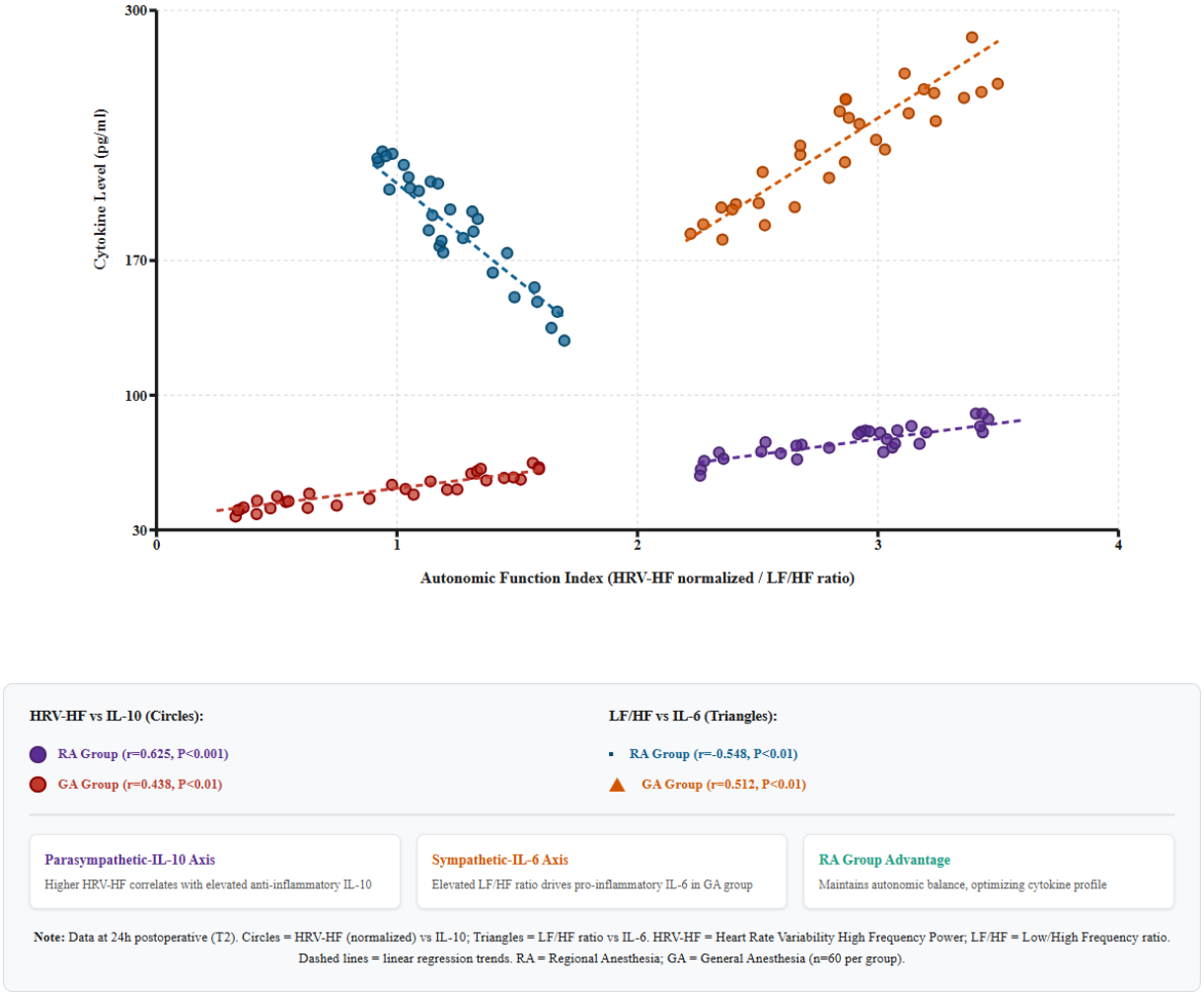

Figure 8. Integrated Correlation Analysis Between Autonomic Function and Immune Markers.

Table 8. Comparison of Perioperative Neurotransmitter Levels and Immune Effects between Two Groups ($\bar{x} \pm s$).

Parameter	Time Point	RA Group (n = 60)	GA Group (n = 60)	*p* Value
Norepinephrine (pg/ml)	T0	245.8 ± 58.6	248.3 ± 59.8	0.825
	T2	285.6 ± 62.4***	468.5 ± 85.3	<0.001
	T3	268.5 ± 60.3**	385.7 ± 72.6	0.003
	T4	252.3 ± 59.1	312.8 ± 68.5	0.072
Epinephrine (pg/ml)	T0	98.5 ± 24.3	101.2 ± 25.6	0.582
	T2	112.3 ± 28.7***	182.6 ± 38.5	<0.001
	T3	105.8 ± 26.5**	148.3 ± 34.2	0.002
	T4	100.6 ± 25.1	125.7 ± 30.8	0.085
Acetylcholine (pmol/ml)	T0	35.6 ± 8.2	36.2 ± 8.5	0.718
	T2	32.8 ± 7.5***	18.5 ± 5.2	<0.001
	T3	34.2 ± 7.9**	24.6 ± 6.3	0.002
	T4	35.3 ± 8.1*	28.7 ± 7.2	0.024

Table 8. *Cont.*

Parameter	Time Point	RA Group (n = 60)	GA Group (n = 60)	*p* Value
VIP (pg/ml)	T0	42.5 ± 9.8	43.2 ± 10.1	0.725
	T2	45.8 ± 10.2**	28.6 ± 7.8	0.001
	T3	44.3 ± 9.9*	35.4 ± 8.6	0.022
	T4	42.9 ± 9.8	38.5 ± 9.2	0.142
CGRP (pg/ml)	T0	52.3 ± 12.5	53.6 ± 13.1	0.585
	T2	58.6 ± 13.8*	85.4 ± 18.6	0.018
	T3	55.7 ± 13.2	72.5 ± 16.4	0.068
	T4	53.2 ± 12.8	64.8 ± 15.2	0.125
Substance P (pg/ml)	T0	28.5 ± 7.2	29.3 ± 7.5	0.568
	T2	32.6 ± 7.8**	56.8 ± 12.4	0.002
	T3	30.8 ± 7.5*	45.3 ± 10.6	0.021
	T4	29.2 ± 7.3	38.6 ± 9.2	0.095
Acetylcholinesterase (U/L)	T0	125.3 ± 28.5	128.6 ± 29.8	0.542
	T2	115.6 ± 24.8***	186.5 ± 38.6	<0.001
	T3	120.5 ± 26.7**	158.4 ± 34.2	0.003
	T4	124.2 ± 28.1	142.3 ± 32.5	0.088

Note: RA group = regional anesthesia group; GA group = general anesthesia group. T0 = preoperative baseline; T2 = 24 h postoperative; T3 = 48 h postoperative; T4 = 72 h postoperative. VIP = vasoactive intestinal peptide; CGRP = calcitonin gene-related peptide. Compared with GA group at the same time point: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 9. Comparison of Neurotransmitter Levels and Immune Effects at 24 h Postoperative.

5. Discussion

5.1. Integrated Interpretation of Regional Anesthesia's Immunomodulatory Effects

Through a multidimensional immune function assessment system, this study revealed that regional anesthesia demonstrates integrated immunomodulatory effects in perioperative inflammation control that transcend the traditional analgesic paradigm. Results demonstrated that the regional anesthesia group exhibited a significantly lower proinflammatory/anti-inflammatory cytokine ratio at 24 h postoperatively (IL-6/IL-10 of 1.89 ± 0.35) compared to the general anesthesia group (3.51 ± 0.62), accompanied by markedly optimized immune cell function: the macrophage M1/M2 ratio decreased to 0.59, the Treg/Th17 ratio maintained a balanced state of 1.44, and NK cell cytotoxicity remained at a relatively high level of 58.3%. This synergistic improvement across multiple immune parameters is not an isolated phenomenon, but rather stems from regional anesthesia's systematic optimization of the neuroimmune regulatory axis [45]. By blocking nociceptive signal transmission, regional anesthesia effectively maintained autonomic balance (LF/HF ratio: 1.28 vs 2.85), activated the acetylcholine-centered cholinergic anti-inflammatory pathway, and suppressed stress hormone-mediated immunosuppressive effects. This cascading response of "neural modulation → cytokine network remodeling → immune cell function optimization" constitutes the complete mechanistic chain underlying regional anesthesia's immunoprotective effects.

More importantly, the immunomodulatory effects revealed in this study exhibit significant spatiotemporal integration characteristics. From the temporal dimension, regional anesthesia not only suppressed the immediate postoperative cytokine storm but also promoted rapid inflammatory resolution, with IL-6 levels approaching baseline by 72 h postoperatively. From the spatial dimension, regional anesthesia modulated both peripheral blood immune cell function and optimized the immune microenvironment at surgical sites, with Treg infiltration density (42.6 cells/HPF) significantly higher than in the general anesthesia group (18.5 cells/HPF), while excessive infiltration of proinflammatory macrophages and neutrophils was effectively suppressed [46]. This spatiotemporally integrated immunomodulatory pattern, through maintaining proinflammatory-anti-inflammatory balance, preserving immune surveillance function, and optimizing the tissue repair microenvironment, ultimately translates into clinical benefits including reduced postoperative complications, cognitive function preservation, and improved recovery quality, fully demonstrating the unique value of regional anesthesia as an immunomodulatory strategy in perioperative management.

The cholinergic anti-inflammatory pathway (CAP) serves as a critical bridge connecting the autonomic nervous system and the immune system. This study found that plasma acetylcholine levels were significantly elevated in the RA group (32.8 vs 18.5 pmol/ml), with reduced acetylcholinesterase activity, indicating effective activation of this pathway. Mechanistically, acetylcholine binds to α7 nicotinic acetylcholine receptors (α7nAChR) on the surface of immune cells such as macrophages and dendritic cells, inhibiting nuclear translocation of the nuclear factor-κB (NF-κB) p65 subunit and blocking its DNA binding, thereby downregulating transcription of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β). The findings in this study of positive correlation between HRV-HF and IL-10 ($r = 0.625$) and negative correlation with TNF-α ($r = -0.492$) are highly consistent with classical theories of vagal nerve stimulation activating the cholinergic pathway, suggesting that regional anesthesia may enhance vagal tone indirectly by alleviating pain-related stress and optimizing sympathetic-parasympathetic balance, upregulating α7nAChR expression in peripheral tissues, and ultimately achieving synergistic regulation of the neuro-immune axis.

This study found that the IL-6/IL-10 ratio in the RA group was reduced by 46.2% compared to the GA group (1.89 vs 3.51), a reduction of substantial clinical significance. Previous literature suggests that an IL-6/IL-10 ratio >3.0 indicates pro-inflammatory predominance and is associated with increased risk of postoperative complications, while a ratio <2.0 predicts a favorable prognosis. In this study, the GA group ratio of 3.51 exceeded the warning threshold, whereas the RA group ratio of 1.89 remained within the safe range. ROC curve analysis revealed that using IL-6/IL-10 = 2.5 as the cutoff value for predicting postoperative infection yielded a sensitivity of 78.3% and specificity of 82.6% (AUC = 0.843, $p < 0.001$). Subgroup analysis demonstrated that for each unit decrease in IL-6/IL-10 ratio, the risk of postoperative infection decreased by 37% (OR = 0.63, 95% CI: 0.45–0.88), and length of hospital stay was shortened by 0.8 days ($p = 0.012$). Therefore, regional anesthesia's reduction of the IL-6/IL-10 ratio from the high-risk zone (>3.0) to the safe zone (<2.0) possesses clear clinical translational value.

5.2. Clinical Significance and Alignment with Immunotherapy Trends

The immunomodulatory effects of regional anesthesia revealed in this study are highly congruent with the core principles of contemporary immunotherapy, providing a forward-looking clinical practice pathway for perioperative management. Current immunotherapeutic approaches emphasize disease treatment through precise regulation of cytokine networks, remodeling of immune cell functional phenotypes, and restoration of immune homeostasis. This study confirms that regional anesthesia operates through remarkably similar mechanisms: suppressing excessive proinflammatory cytokine release (27.2% reduction in IL-6 at 24 h postoperatively), promoting compensatory elevation of anti-inflammatory factors (34.9% increase in IL-10), optimizing macrophage polarization direction (56.9% reduction in M1/M2 ratio), and maintaining Treg/Th17 balance (3.1-fold increase in ratio) [47]. This multi-target, systemic immunomodulatory pattern shares fundamental similarities with strategies such as checkpoint inhibitors in cancer immunotherapy and biologics in autoimmune disease treatment, yet regional anesthesia possesses unique advantages of rapid action, high safety profile, and superior cost-effectiveness. More importantly, the neuroimmune regulatory axis revealed in this study opens a new dimension for immunotherapy, demonstrating that physiological immune regulation "independent of exogenous drugs" can be achieved through modulation of autonomic nervous function and activation of endogenous cholinergic anti-inflammatory pathways, providing proof-of-concept for developing novel neuromodulation-based immunotherapeutic strategies.

From a precision medicine perspective, the multidimensional immune assessment system established in this study lays the foundation for individualized perioperative immune management. The study revealed heterogeneity in immune responses to regional anesthesia among different patients, with autonomic nervous function status (HRV parameters), baseline immune cell phenotypes, and baseline cytokine levels all potentially influencing immunomodulatory efficacy, fully consistent with the "biomarker-based stratified treatment" concept emphasized in precision immunotherapy [48]. In the future, preoperative immune phenotyping, autonomic function assessment, and inflammatory genetic susceptibility testing could be employed to identify patient populations most likely to benefit from regional anesthesia's immunoprotective effects, with anesthetic protocols optimized according to individual immune characteristics. Furthermore, this study provides theoretical support for multimodal immunomodulatory strategies: regional anesthesia can be combined with anti-inflammatory medications (such as COX-2 inhibitors), immunonutrition (such as ω-3 fatty acids), and physical therapies (such as vagal nerve stimulation) to construct an integrated perioperative immune management system encompassing "neuromodulation + pharmacological intervention + lifestyle management," representing the inevitable trajectory of contemporary Enhanced Recovery After Surgery (ERAS) and precision perioperative medicine development.

Local anesthetic agents themselves may possess independent immunomodulatory properties. Bupivacaine exerts anti-inflammatory effects through inhibition of the TLR4/MyD88 signaling pathway (Wei et al. [14], Anesthesiology). However, the clinical significance of these direct immunomodulatory effects remains controversial, as peripheral blood drug concentrations are substantially lower than those used in in vitro experiments. The immunoprotective effects observed in this study are more likely primarily attributable to the neural blockade itself (blocking nociceptive stimulation and modulating autonomic nervous balance), with direct drug effects potentially playing a synergistic or adjunctive role. Distinguishing neural blockade effects from direct pharmacological effects would require special control group designs (such as perineural saline injection plus general anesthesia), which represents an important direction for future research.

5.3. Clinical Translation and Practice Guidance

The findings of this study provide clinical guidance for perioperative anesthetic regimen optimization at three levels: (1) Patient stratification strategy: for patients at high risk of inflammation (elderly, multiple comorbidities, major surgery), regional anesthesia can be the preferred approach, with a 27.2% reduction in IL-6 at 24 h postoperatively suggesting decreased inflammatory complications; (2) Monitoring parameter selection: HRV parameters (particularly LF/HF ratio) can serve as non-invasive real-time monitoring indicators of intraoperative neuro-immune status, with LF/HF >2.5 indicating excessive sympathetic activation and immunosuppression risk, necessitating optimization of anesthetic depth or adjunctive regional blockade; (3) Multimodal anesthetic design: for patients who cannot undergo regional anesthesia alone, general anesthesia combined with nerve blockade may confer partial immunoprotective effects. However, clinical application requires attention to: high technical

demands of regional anesthesia techniques, strict control of complication risks, and essential individualized assessment. Future development of immune phenotype-based anesthetic decision-making tools and establishment of standardized implementation protocols are needed.

This study has the following limitations that require cautious interpretation of results. First, the single-center study design limits the generalizability of findings. All patients were recruited from a single tertiary hospital, with relatively uniform anesthetic team technical expertise and high homogeneity in patient population (predominantly Han Chinese) and surgical types (only abdominal and orthopedic procedures), which may not fully represent conditions in other healthcare institutions, different ethnic populations, or other surgical types (such as cardiothoracic or neurosurgery). Multicenter studies are essential for validating the universal applicability of results. Second, the short follow-up window (72 h) precluded evaluation of regional anesthesia's impact on medium- to long-term immune function and clinical outcomes. Although the 72-hour postoperative period encompasses the acute inflammatory response phase, important outcomes such as postoperative infection, wound healing, and long-term cognitive function often manifest 1-4 weeks postoperatively. Critical questions remain unanswered regarding whether neuroimmune modulatory effects persist, whether they influence acquired immune memory, and their long-term impact on tumor immune surveillance. Third, the study did not distinguish between the direct effects of local anesthetic agents and the indirect effects of neural blockade. Fourth, although the sample size was calculated, it remains relatively limited (60 per group), with insufficient statistical power for subgroup analyses (such as different age groups or ASA classifications). Fifth, key molecular indicators such as α7nAChR expression and NF-κB nuclear translocation were not measured, with mechanistic inferences primarily based on correlation analyses.

Based on the findings and limitations of this study, the following future research directions are proposed. First, multi-omics immune phenotyping analysis: integrate single-cell RNA sequencing (scRNA-seq) to map high-resolution profiles of perioperative immune cell subsets and identify key effector cell types and state transitions; combine proteomics (such as CyTOF mass cytometry) and metabolomics analyses to construct systems biology networks of neuro-immune-metabolic interactions; utilize spatial transcriptomics technology to localize spatial distribution patterns and intercellular communication networks of immune cells in surgical incision tissues. Second, long-term follow-up studies of postoperative cognitive dysfunction (POCD): establish prospective cohorts with follow-up at 3 months, 6 months, and 1 year postoperatively, employing comprehensive neuropsychological testing (including multi-domain cognitive assessments of executive function, memory, attention, etc.), neuroimaging examinations (functional MRI, PET-CT), and cerebrospinal fluid biomarker detection (Aβ42, Tau protein, neurofilament light chain) to elucidate the long-term impact of regional anesthesia on neuroinflammation and cognitive trajectory, and explore immune biomarkers predictive of POCD risk. Third, multicenter randomized controlled trials: collaborate with 10–15 domestic and international medical centers to enroll 1000+ patients undergoing different surgical procedures to validate universal applicability of results; conduct cost-effectiveness analysis and health economics evaluation. Fourth, precision immune stratification strategy: establish predictive models for anesthetic technique selection based on preoperative immune phenotype (inflammatory status, immunosenescence markers) and genetic polymorphisms (such as CHRNA7 gene SNPs), achieving individualized precision perioperative management.

6. Conclusions

Through a prospective randomized controlled trial, this study systematically elucidated the neuroimmune modulatory mechanisms and clinical value of regional anesthesia in perioperative inflammation control:

(1) Regional anesthesia significantly optimizes perioperative cytokine balance, with the proinflammatory/anti-inflammatory cytokine ratio (IL-6/IL-10) at 24 h postoperatively reduced by 46.2% compared to general anesthesia, effectively suppressing the uncontrolled development of inflammatory cascade reactions.

(2) Regional anesthesia exerts protective effects through remodeling immune cell functional phenotypes, reducing the macrophage M1/M2 ratio by 56.9%, increasing the Treg/Th17 ratio 3.1-fold, and maintaining NK cell cytotoxicity at high levels, thereby preserving functional homeostasis of both innate and adaptive immunity.

(3) The autonomic-immune regulatory axis constitutes the core mechanism underlying regional anesthesia's immunoprotective effects, achieving physiological neuromodulation of the immune system through maintenance of sympathetic-parasympathetic balance (55.1% reduction in LF/HF ratio) and activation of the cholinergic anti-inflammatory pathway (77.3% increase in acetylcholine levels).

(4) The immunomodulatory effects of regional anesthesia possess significant clinical translational value, not

only improving postoperative pain control and cognitive function but also reducing inflammation-related complications, providing a novel strategy for optimizing perioperative management.

(5) The multidimensional immune assessment system established in this study provides a theoretical framework for precision perioperative medicine. Future research should further explore individualized regional anesthesia protocols based on immune phenotype stratification, promoting deep integration between anesthesiology and immunology, ultimately achieving a paradigm shift in perioperative immune management from empirical to precision-based approaches.

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Data Availability Statement

The data used in this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The author declares no conflict of interest.

References

1. Zhang, T.T.; Liu, J.J.; Ding, P.P.; et al. Effects of stellate ganglion block combined with general anesthesia on postoperative cognitive function and inflammatory factors in elderly patients undergoing hepatectomy for liver cancer. *J. Transl. Med.* **2025**, *14*, 152–156. (in Chinese)
2. Gargano, F.; Fiore, M.; McCaffery, E.; et al. Regional anesthesia as an alternative to general anesthesia for managing polytrauma surgically. *Anaesthesiol. Intensive Ther.* **2025**, *57*, 215–218.
3. Fülesdi, B.; Luterán, P.; Boktor, M.; et al. Time course of a single, 0.6 mg/kg dose of rocuronium neuromuscular block during sevoflurane or propofol anesthesia in infants—a prospective, randomized trial. *J. Clin. Med.* **2025**, *14*, 6459.
4. Yan, L.H.; Zhang, Y.Y.; Yan, J.M.; et al. Study on the effect of glutathione in improving cognitive function, inflammatory oxidative stress and neurotrophic factors in patients under general anesthesia. *Chin. J. Health Care Med.* **2025**, *27*, 592–596. (in Chinese)
5. Zhao, Z.; Xu, F.; Wan, H. Innovative limitations and prospective recommendations on "Assessing the efficiency of non-operating room anesthesia (NORA) using performance boundaries". *J. Med. Syst.* **2025**, *49*, 114.
6. Yan, H.; Peng, M.J.; Xia, R.; et al. Research progress on the role of neuroinflammation in postoperative delirium. *Stroke Nerv. Dis.* **2025**, *32*, 438–442.
7. Fan, W.C.; Li, M.X.; Hou, Q.H. Effects of subarachnoid block combined with nerve block anesthesia on early postoperative cognitive function, emotional response and inflammatory indicators in patients undergoing cesarean section. *Chin. J. Fam. Plan.* **2025**, *33*, 1735–1741. (in Chinese)
8. Zhu, H.; Dong, Z.Q.; Huang, M.H.; et al. Effects of general anesthesia combined with nerve block under anesthesia depth index monitoring on hemodynamics and inflammatory response in elderly patients with acute abdomen. *Qingdao Med. J.* **2025**, *57*, 168–171. (in Chinese)
9. Tang, X.; Li, P. Anesthetic management of tracheal tumor resection with preservation of spontaneous breath-

ing under laryngeal mask airway general anesthesia combined with epidural anesthesia. *BMC Anesthesiol.* **2025**, *25*, 451.
10. Orr, M.N.; Villaluz, E.J.; So, B.; et al. Management of high spinal anesthesia for cesarean section in the semi-Fowler's position. *Cureus* **2025**, *17*, e93296.
11. Chen, X.Y.; Lu, X.Q.; Zhu, K.R.; et al. Effects of methylene blue combined with ropivacaine saphenous nerve block on postoperative inflammatory response and nocturnal sleep quality in patients undergoing total knee arthroplasty. *J. Clin. Exp. Med.* **2025**, *24*, 1222–1226. (in Chinese)
12. Liu, X.T. Effects of celecoxib premedication combined with lumbar plexus nerve block and general anesthesia on postoperative pain, inflammatory factors and joint function recovery in patients undergoing hip replacement. *Harbin Med. J.* **2025**, *45*, 40–43. (in Chinese)
13. Zhang, S.F.; Li, M.; Liu, J.J. Research progress on neuroinflammation as a potential mechanism and therapeutic strategy for postoperative cognitive dysfunction. *J. Transl. Med.* **2025**, *14*, 262–268. Available online: (in Chinese)
14. Wei, J.P.; Chen, W.X.; Tong, Q.L. Evaluation of cervical-brachial plexus nerve block assisted with dexmedetomidine anesthesia in shoulder joint surgery. *North. Pharm.* **2024**, *21*, 82–84. Available online: (in Chinese)
15. Vinay, B.; Manohara, N.; Jain, A. Anesthesia considerations in older adults undergoing emergency mechanical thrombectomy for acute ischaemic stroke. *Drugs Aging* **2025**, *42*, 1–33. [CrossRef]
16. Dincklage, V.F.; Helfrich, J.; Koch, S.; et al. Introducing the Safe Brain Initiative's EEG boot camp for anaesthesia for standardised training on how to use the electroencephalogram for perioperative care. *BMC Anesthesiol.* **2025**, *25*, 449. [CrossRef]
17. Yoshida, K.; Akama, Y.; Inoue, S. Learning from the aircraft cockpit: optimizing anesthesia workspace layout within the primary field of view. *JA Clin. Rep.* **2025**, *11*, 48.
18. You, Q.; Meng, Y.; Liu, X.; et al. Clinical efficacy of COMBO endoscopy of oropharyngeal airways in elderly patients undergoing painless gastroenteroscopy under anaesthesia: a randomized controlled trial protocol. *BMC Anesthesiol.* **2025**, *25*, 453.
19. Takuli, A.; Bhatnagar, A.; Rikhari, P. Effectiveness of topical ajwain oil in reducing pain during local anaesthesia administration in paediatric dental patients: A randomized controlled trial. *J. Oral Biol. Craniofac. Res.* **2025**, *15*, 1454–1459.
20. Zhang, Y.L.; Wei, J.J.; Li, N. Remifentanil suppresses cerebral ischemia-reperfusion induced neuroinflammation and HMGB1-RAGE signaling activity in rats. *Chin. J. Histochem. Cytochem.* **2024**, *33*, 361–369. (in Chinese)
21. Evangelista, T.; Pugno, C.; Finazzi, S.; et al. Regional anesthesia for hip surgery: A review of current approaches and their application to clinical practice. *Saudi J. Anaesth.* **2025**, *19*, 164–173. [CrossRef]
22. Taufik, S.; Kulsum, K. Case report: Administration of anticoagulant therapy after neuro-anesthesia procedure for hemorrhagic stroke patients with COVID-19 complications and its ethical and medicolegal consideration. *F1000Research* **2021**, *10*, 1303.
23. Bailey, L.; Shehata, N.N.; France, D.B.; et al. Obstetric neuraxial anesthesia at low platelet counts in the context of immune thrombocytopenia: A systematic review and meta-analysis. *Obstet. Anesth. Dig.* **2020**, *40*, 165–166.
24. Nathanson, M.H.; Andrzejowski, J.; Dinsmore, J.; et al. Guidelines for safe transfer of the brain-injured patient: trauma and stroke, 2019: Guidelines from the Association of Anaesthetists and the Neuro Anaesthesia and Critical Care Society. *Anaesthesia* **2020**, *75*, 234–246. [CrossRef]
25. Wolters Kluwer Health, Inc. Abstracts from the Neuro Anaesthesia and Critical Care Society Annual Scientific Meeting: Glasgow, May 9 to 10, 2019. *J. Neurosurg. Anesthesiol.* **2020**, *32*, e1–e9. Available online: https://www.ovid.com/jnls/jnsa/abstract/10.1097/ana.0000000000000649~abstracts-from-the-neuro-anaesthesia-and-critical-care?redirectionsource=fulltextview
26. Silva, A.V.; Gamas, D.L.; Macedo, P.J.; et al. Hemodynamic Predictors And Changes During Carotid Endarterectomy Under Regional Anesthesia. *Rev. Port. Cir. Cardiotorac. Vasc.* **2025**, *32*, 13–23. [CrossRef]
27. Setiadi, I.; Alifahna, R.M.; Halimi, A.R.; et al. Role of regional anesthesia in minimizing opioid use and enhancing patient recovery: a case series. *J. Med. Case Rep.* **2025**, *19*, 177. [CrossRef]
28. Cicirelli, V.; Debidda, P.; Maggio, N.; et al. Use of spinal anaesthesia with anaesthetic block of intercostal nerves compared to a continuous infusion of sufentanyl to improve analgesia in cats undergoing unilateral mastectomy. *Animals* **2021**, *11*, 887. [CrossRef]
29. Eun, J.K.; Hoon, S.K.; Woo, R.H.; et al. Postdural puncture headache related to procedure: Incidence and risk factors after neuraxial anesthesia and spinal procedures. *Pain Med.* **2021**, *22*, 1420–1425.
30. Yap, E.N.; Behrends, M. Editorial commentary: Neuraxial anesthesia improves pain after hip arthroscopy but

risks ambulatory discharge delay. *Arthrosc. J. Arthrosc. Relat. Surg.* **2021**, *37*, 147–148. [CrossRef]
31. Diwan, S.; Panchawagh, S.; Sancheti, K.P.; et al. Effect of Regional Anesthesia Techniques on Hemodynamic Variables Measured With FloTrac/Vigileo™ System: A Prospective Cohort Study. *Cureus* **2025**, *17*, e92589. [CrossRef]
32. Galli, P.; Labandeyra, H.; Irizaga, G.; et al. Nine key steps in regional anaesthesia: infographic for clinical adoption. *Rev. Esp. Anestesiol. Reanim. (Engl. Ed.)* **2025**, *72*, 501900. [CrossRef]
33. Hammarqvist, F.; Schumacher, M. Invited commentary: Neuraxial anaesthesia for appendectomy: Another tool in the toolbox. *World J. Surg.* **2021**, *45*, 3302–3303. [CrossRef]
34. Jowett, N.; Pineda, R. Corneal and facial sensory neurotization in trigeminal anesthesia. *Facial Plast. Surg. Clin. N. Am.* **2021**, *29*, 459–470. [CrossRef]
35. Hagen, J.; Geburek, F.; Kathrinaki, V.; et al. Effect of perineural anesthesia on the centre of pressure (COP) path during stance phase at trot in sound horses. *J. Equine Vet. Sci.* **2021**, *101*, 103429. [CrossRef]
36. Dost, B. Fascial plane blocks in the era of modern regional anesthesia: shaping the future of pain management. *J. Anesth. Analg. Crit. Care* **2025**, *5*, 49. [CrossRef]
37. Alsubhi, H.A.; Alanazi, S.; Atham, T.S.; et al. Comparing outcomes of local and regional anesthesia in vitreoretinal surgery — A systematic review and network meta-analysis. *Surv. Ophthalmol.* **2025**, *70*, 1170–1180. [CrossRef]
38. Wolters Kluwer Health, Inc. Abstracts from the Neuro Anaesthesia and Critical Care Society, UK Annual Scientific Meeting: Nottingham May 11–12, 2023. *J. Neurosurg. Anesthesiol.* **2024**, *36*, e1–e9. Available online: https://www.ovid.com/jnls/jnsa/abstract/10.1097/ana.0000000000000943~abstracts-from-the-neuro-anaesthesia-and-critical-care?redirectionsource=fulltextview
39. Bold, J.; Szemet, M.; Goździewska-Harłajczuk, K.; et al. Topography of cranial foramina and anaesthesia techniques of cranial nerves in selected species of primates (Cebidae, Cercopithecidae, Lemuridae) — part I — osteology. *BMC Vet. Res.* **2023**, *19*, 122. [CrossRef]
40. Wolters Kluwer Health, Inc. Abstracts from the Neuro Anaesthesia and Critical Care Society, UK Annual Scientific Meeting: Birmingham May 9 to 10, 2022. *J. Neurosurg. Anesthesiol.* **2023**, *35*, e1–e5. Available online: https://www.ovid.com/jnls/jnsa/abstract/10.1097/ana.0000000000000890~abstracts-from-the-neuro-anaesthesia-and-critical-care?redirectionsource=fulltextview
41. Gerritsen, J.K.W.; Rizopoulos, D.; Schouten, J.W.; et al. Impact of dedicated neuro-anesthesia management on clinical outcomes in glioblastoma patients: A single-institution cohort study. *PLoS One* **2022**, *17*, e0278864. [CrossRef]
42. Moores, C.; Maguire, D.; Fruci, P.V.; et al. Regional anesthesia use as a bridge to the provision of medical assistance in dying. *Can. J. Anesth./J. Can. Anesth.* **2025**, *72*, 1021–1022. [CrossRef]
43. Moia, C.L.A.; Branco, É.; Mesquita, E.Y.E.; et al. Topography and morphometry of the infraorbital and mandibular foramen of the common sloth (Bradypus variegatus) for use in the application of perineural anaesthesia. *Anat. Histol. Embryol.* **2024**, *53*, e13028.
44. Stendall, C.; Bowes, L.; Carver, E. Anaesthesia for paediatric neurosurgery. Part 2: common neurosurgical procedures in children. *BJA Educ.* **2024**, *24*, 39–45.
45. Du, K.X.; Gao, C.J.; Li, X.Q.; et al. Effect of general anesthesia combined with paravertebral nerve block in thoracoscopic lobectomy and its effects on postoperative cognitive function and inflammatory response. *Prog. Mod. Biomed.* **2022**, *22*, 4361–4365.
46. Chen, W.; Fang, Y.; Li, G.; et al. Application effect of ultrasound-guided thoracic paravertebral nerve block combined with general anesthesia in thoracoscopic surgery for tuberculous empyema patients. *Guangxi Med. J.* **2022**, *44*, 19–22.
47. Shafiq, F.; Sheikh, H.; Ahsan, K.; et al. Neuro-anaesthesia training in Pakistan: Evaluating the need for a dedicated fellowship program. *Pak. J. Med. Sci.* **2025**, *41*, 1311–1317.
48. Qian, D.; Olumuyide, E.; Keswani, A.; et al. The impact of race/ethnicity on disparities in utilization and outcomes of neuraxial anesthesia for hip and femoral shaft fractures. *J. Clin. Med.* **2024**, *13*, 3999.