Impact of the CD40-CD40L system on pain severity in children after anterior abdominal wall surgery using various anaesthesia techniques

Abstract. Background. The aim of the study was to assess changes in the serum CD40L level and its potential relationship with pain severity in children after anterior abdominal wall surgery on the background of general anaesthesia and its combination with various regional anaesthesia techniques. Materials and methods. The study included 87 children who underwent anterior abdominal wall surgery using different analgesic techniques. All children were divided into 3 groups: group I (n = 33) — general anaesthesia using morphine; group II (n = 27) — general anaesthesia with the transversalis fascia plane block (TFPB); group III (n = 27) — general anaesthesia using the TFPB combined with the quadratus lumborum block (QLB-4) via a single injection. Results. In group I, the mean serum level of CD40L two hours after surgery was 4,283 pg/ml, with a slight downward trend at discharge (Mann-Whitney U test = 52.5; p = 0.593). In group II, CD40L reduced significantly, by 25.3 % (U = 10.0; p = 0.002); 24 hours after surgery, it decreased by 15.1 % (U = 26.0; p = 0.20). At discharge, the level of CD40L in this group reduced significantly, by 54.4 % (U = 7.0; p = 0.003). In group III during all observation periods, the mean serum level of CD40L was 4—7.6 times lower (U = 0.0; p = 0.000) than corresponding levels in children of group II. Conclusions. The indicators of CD40L signaling were found to increase in paediatric anterior abdominal wall surgeries. There was a close positive correlation between postsurgical pain severity and CD40L serum levels. Single-injection TFPB + QLB-4 resulted in the lowest serum CD40L levels, an indicative of the lowest intensity of postsurgical pain. Keywords: regional analgesia; CD40L; postsurgical pain; children; pain management

Introduction. The CD40-CD40L system involved in the regulation of immunological processes, including T cell activation, immunoglobulin and cytokemade production, has recently attracted increasing attention. Impaired CD40 signaling is involved in the pathogenesis of many pathological processes associated with the development of the inflammatory response and thrombosis [1, 2].

CD40 and CD40 ligand (CD40L) are known as type I and II transmembrane proteins belonging to the tumor necrosis factor superfamily, which exist in both membrane-bound and soluble forms. CD40 is expressed on both immune (B cells, dendritic cells, monocytes, and macrophages) and non-immune cells (endothelial cells, vascular smooth muscle cells, and fibroblasts). In the central nervous system, CD40 is expressed by microglia, while neurons and astrocytes are not capable of producing this protein. CD40L is mainly expressed on activated T cells and platelets in response to infectious and non-infectious inflammatory processes. If CD40L interacts with CD40 receptor, trimerization of the latter occurs, which leads to its activation. The activation of CD40 receptor stimulates the secretion of growth factors, chemokines, and pro-inflammatory cytokines through a variety of intracellular signal transduction pathways [2, 3].
Experimental models for the study of peripheral nerve damage have demonstrated that the CD40–CD40L pathway is involved in the generation of neuropathic pain associated with increased expression of C-C motif ligand 2 and calcitonin gene-related peptide [4–9].

However, literature data on the pronociceptive effects of the CD40–CD40L pathway are scarce and its role in pain generation in practical medicine, especially anaesthetic practice, has not been studied at all. Pain is one of the earliest psychophysical functions to be formed; by the 30th week of pregnancy, all the pathways of pain transduction and perception are already formed; thus, both fetus and child are able to perceive pain and its intensity is often even greater than in adults. Some experts erroneously believe that infants do not feel pain as their central nervous system is still immature [10]. Abdominal pain is an important adaptive response of the body which sends an alarm signal in response to harmful stimuli and enables us to choose the most appropriate behaviour for the situation. Nevertheless, despite its protective function, pain is one of the clinical symptoms of the disease. Acute pain requires the cause to be determined, emergency to be excluded, and appropriate care to be provided, while in case of chronic pain, it is difficult to eliminate the cause, which is usually already known, and the main goal is to provide an adequate pain relief, which sometimes can be quite difficult [11].

Post-surgical pain in children is one of the main factors determining the child’s condition after surgery and contributing to complication development; therefore, pain management in the postoperative period is one of the main goals of intensive care. In paediatrics, regional anaesthesia (RA) is one of the most valuable and safest means of perioperative pain management, being an essential part of modern anaesthetic practice [12]. Novel RA techniques, especially the anterolateral and the posterolateral trunk blocks, are quite promising today. The benefits of RA in children include: accelerated recovery; decreased opioid use; reduced incidence of postoperative nausea and vomiting; decreased postoperative pain severity; reduced incidence of respiratory complications; decreased healthcare system costs [13–16]. The quadratus lumborum block (QLB) is recommended for surgeries during which both the somatic and visceral components of pain should be affected, including caesarean section [17], gynaecological surgical procedures such as hysterectomy [18], small bowel resection [19], large bowel resection [20], nephrectomy, colostomy closure, appendectomy [21], gastrectomy, hernia repair [22]. Cases of QLB application after bifenomaral shunting and cholecystectomy have been described as well [23]. The transversalis fascia plane block (TFPB) is a truncal block that targets the L1 nerve branches, namely the ilioinguinal and iliohypogastric nerves, where they emerge from the lateral border of the psoas major muscle, inferior to the 12th rib; the TFPB was first proposed by Hebbard in 2009 [24–27].

The aim of the study was to assess changes in the serum CD40L level and its potential relationship with pain severity in children after anterior abdominal wall surgery on the background of general anaesthesia and its combination with various RA techniques.

**Materials and methods**

The study included 87 children (46 boys and 41 girls) aged 7–18 years who were treated at the surgical department of a Communal Non-Profit Enterprise “Ivano-Frankivsk Regional Children’s Clinical Hospital of Ivano-Frankivsk Regional Council” (Ivano-Frankivsk, Ukraine) and underwent anterior abdominal wall surgery for inguinal hernia, appendicitis using different analgesic techniques in 2020–2022. Inclusion criteria were inguinal hernia and appendicitis ASA I–II, age of 7–18 years, mandatory parental consent to involve their child in clinical research. Exclusion criteria: age under 7 years; ASA grade III or higher, mental disorders, neoplasms, or tumours, acute or inflammatory processes of any aetiology and localization, sepsis, shock; previous lower abdominal surgery; pain for six months prior to surgery; refusal to participate in the research; children whose parents refused to give consent and children who gave no consent.

All patients were divided into 3 groups: group I (n = 33) — general anaesthesia using morphine; group II (n = 27) — general anaesthesia with the TFPB; group III (n = 27) — general anaesthesia using the TFPB combined with the QLB-4 via a single injection. All clinical and laboratory studies were conducted in accordance with the World Medical Association Declaration of Helsinki — Ethical Principles for Medical Research Involving Human Subjects. According to the law, prior to a subject’s participation in the study, a written informed consent form was signed by each patient (parents/adult guardians). The manuscript was approved by the Ethics Committee of the Communal Non-Profit Enterprise “Ivano-Frankivsk Regional Children’s Clinical Hospital of Ivano-Frankivsk Regional Council” as evidenced by an Excerpt from the Minute of the Committee Meeting No. 2 dated February 24, 2022.

Statistical analysis was carried out on a personal computer using statistical software packages MS Excel, SPSS/22 for Windows. The results obtained are presented as M ± m. The Shapiro-Wilk test was used for a normal distribution. The non-parametric Mann-Whitney U test was applied to compare differences between the indicators (if the outcome was not normally distributed). The relationship between the indicators was determined using Spearman’s correlation. In addition, the percentile method was used (median P5, P10, P25, P50, P75, P90, P95 were determined). Differences were considered statistically significant at p < 0.05.

**Results**

First, changes were assessed in the serum CD40L level after anterior abdominal wall surgery on the background of general anaesthesia and its combination with various RA techniques (Table 1). In children receiving general opioid anaesthesia, the serum level of CD40L was high 2 hours after surgery; its median was 4,283 pg/ml with the interquartile range (P25–P75) of 4,024–4,583 pg/ml. One day after surgery, the mean serum concentration of CD40L reduced as compared to that observed 2 hours after surgery; however, there were no statistically significant differences (U = 52; p = 0.563). At discharge, the mean serum level of
CD40L was still high and did not significantly differ from that observed 24 hours after surgery (У = 52.5; р = 0.593). In patients receiving general anaesthesia combined with the TFPB, the mean serum concentration of CD40L reduced significantly, by 25.3 % (У = 10.0; р = 0.002) two hours after surgery, as compared to that in general anaesthesia alone: the median was 3,135 pg/ml and the interquartile range (P25-P75) was 2,440–3,587 pg/ml. Twenty-four hours after surgery, the mean serum level of CD40L reduced by 15.1 % (У = 26.0; р = 0.20) as compared to that observed 2 hours after surgery and by 33.3 % (У = 10.0; р = 0.003) as compared to that in patients receiving general opioid anaesthesia alone. At discharge, the mean serum level of CD40L reduced significantly, by 54.4 % (У = 7.0; р = 0.003) as compared to that seen during the previous observation period in this group and by 68.5 % (У = 3.0; р = 0.000) as compared to the corresponding indicator when using general anaesthesia. In children receiving general anaesthesia combined with single-injection intra-muscular TFPB + QLB-4, during all observation periods, the mean serum CD40L was 4–7.6 times lower (У = 0.0; р = 0.000) than corresponding levels in the group of general anaesthesia combined with the TFPB. Twenty-four hours after surgery, the mean serum concentration of CD40L reduced significantly, by 39.6 % (U = 12.0; р = 0.012) as compared to that observed two hours after surgery. The lowest serum level of CD40L was recorded at discharge: the median was 133 pg/ml, and the interquartile range (P25-P75) was 69.4–188 pg/ml.

The results obtained showed that single-injection TFPB + QLB-4 resulted in the lowest mean serum level of CD40L, which was significantly different from those observed with other types of anaesthesia.

### Discussion

The relationship between the serum level of CD40L and pain severity in children after surgery using different types of anaesthesia was assessed as well (Table 2). The correlation and percentile analyses provided relevant evidence of CD40 pathway involvement in the development of postsurgical pain in children receiving different anaesthesia. Between the serum level of CD40L and the mean score of pain severity on the visu2analogue scale (VAS), a statistically significant and moderate positive correlation was found.

The percentile analysis showed that pain severity on the VAS was the lowest with low serum levels of CD40L (corresponds to the interquartile range of P0–P25). Pain severity was significantly higher with moderate serum concentration of CD40L (corresponds to the P25–P75 interquartile range): the mean VAS score was 68 % higher than that with low serum CD40L concentrations. A high serum CD40L (corresponds to the P75–P100 interquartile range) was accompanied by maximum pain severity — the mean VAS score exceeded the corresponding indicator for moderate serum CD40L concentration by 40 % and was 2.4 times higher than that with low serum CD40L levels.

### Conclusions

The results of our study provided evidence of CD40L signaling involvement in the development of postsurgical pain in children receiving different types of anaesthesia. The indicators of CD40L signaling were found to increase in paediatric anterior abdominal wall surgeries. A combination of conventional anaesthesia and RA techniques resulted in significantly lower serum CD40L levels as compared to the group which received only general anaesthesia. There was a close positive correlation between postsurgical pain severity and CD40L serum levels. Single-injection TFPB + QLB-4 resulted in the lowest serum CD40L levels, an indicative of the lowest intensity of postsurgical pain.

### Table 1. Changes in the serum level of CD40L in children after anterior abdominal wall surgery using general anaesthesia and its combination with various regional anaesthesia techniques, pg/ml

<table>
<thead>
<tr>
<th>Group</th>
<th>Type of anaesthesia</th>
<th>n</th>
<th>Observation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 h after surgery</td>
</tr>
<tr>
<td>1</td>
<td>General anaesthesia</td>
<td>33</td>
<td>4.191 ± 160</td>
</tr>
<tr>
<td>2</td>
<td>General anaesthesia + TFPB</td>
<td>27</td>
<td>3.130 ± 248*</td>
</tr>
<tr>
<td>3</td>
<td>General anaesthesia + TFPB + QLB-4</td>
<td>27</td>
<td>758.0 ± 81.2**</td>
</tr>
</tbody>
</table>

Notes: п < 0.05 compared: * — to the corresponding indicator in patients receiving general anaesthesia alone during a particular observation period; ** — to the corresponding indicator when using general anaesthesia and RA techniques resulted in significantly lower serum CD40L levels as compared to the group which received only general anaesthesia. There was a close positive correlation between postsurgical pain severity and CD40L serum levels. Single-injection TFPB + QLB-4 resulted in the lowest serum CD40L levels, an indicative of the lowest intensity of postsurgical pain.

### Table 2. Relationship between serum CD40L level and pain severity in children after anterior abdominal wall surgery using general anaesthesia and its combination with various regional anaesthesia techniques

<table>
<thead>
<tr>
<th>Indicator</th>
<th>P0-P25 &lt; 671 pg/ml</th>
<th>P25-P75 671–3,971 pg/ml</th>
<th>P75-P100 &gt; 3,971 pg/ml</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean VAS score</td>
<td>3.09 ± 0.17</td>
<td>5.19 ± 0.18</td>
<td>7.27 ± 0.12</td>
<td>( r_s ) = 0.62</td>
</tr>
<tr>
<td>p</td>
<td>—</td>
<td>( p_{0.5} &lt; 0.01 )</td>
<td>( p_{0.3} &lt; 0.01 )</td>
<td>( p &lt; 0.001 )</td>
</tr>
</tbody>
</table>

Note: the subscripts below п indicate the numbers of groups between which the significance of differences was assessed.
References


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Вплив сигнальної системи CD40-CD40L на інтенсивність болю в дітей після операцій на передній черевній стінці при використанні різних методик знеболювання

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В експериментальних дослідженнях на моделях пошкодження периферичних нервів показано, що сигнальна система CD40-CD40L відіграє важливу роль у розвитку нейропатичного болю. Регіонарна анестезія в педіатричній практиці є одним з найбільш цінних і безпечних засобів для лікування періопераційного болю, а також важливою частиною сучасної анестезіологічної практики.

Мета: оцінити динаміку вмісту ліганду CD40L у сироватці крові та його можливий зв’язок з інтенсивністю болю в дітей на тлі загального знеболювання та його комбінації з різними варіантами регіонарної анестезії після операцій на передній черевній стінці.

Матеріали та методи. У дослідженні взяли участь 87 дітей віком 7–18 років, яким проводилось оперативне втручання на передній черевній стінці із різними варіантами анестезіологічного знеболювання. Усі пацієнти були розподілені на 3 групи: І (n = 33) — загальне знеболювання з використанням морфіну; ІІ (n = 27) — загальне знеболювання із застосуванням регіонарного блоку поперечної фасції живота (РБПФЖ); ІІІ (n = 27) — загальне знеболювання із застосуванням РБПФЖ в поєднанні з блокадою квадратного м’яза попереку (КМП-4) однією ін’єкцією.

Результати. Установлено, що в дітей І групи через дві години після оперативного втручання вміст CD40L в сироватці крові становив 4283 пг/мл, з незначною тенденцією до зниження на момент виписки (U-критерій Манна — Уїтні = 52,5; р = 0,593). Пацієнти ІІ групи через 2 год після операції мали вірогідно менший середній рівень CD40L в сироватці крові — на 25,3 % (U = 10,0; р = 0,002) порівняно з дітьми І групи. Через добу після операції середній сироватковий уміст CD40L був меншим на 15,1 % (U = 26,0; р = 0,20) порівняно з таким станом на 2 год, а також статистично вірогідно меншим на 33,3 % (U = 10,0; р = 0,003) відносно показника при використанні лише загального опіоїдного знеболювання в той же термін дослідження. На момент виписки середній рівень CD40L у сироватці крові був вірогідно меншим — на 54,4 % (U = 7,0; р = 0,003) відносно попереднього терміну дослідження в цій групі, а також на 68,5 % (U = 3,0; р = 0,000) порівняно з відповідним показником у групі загального знеболювання.

Висновки. Установлено, що показники сигнальної системи CD40L мають тенденцію до зростання при операційних втручаннях на передній черевній стінці у дітей. Інтенсивність післяопераційного болю має тісний прямий зв’язок із рівнем CD40L у сироватці крові.

Ключові слова: регіонарна аналгезія; CD40L; післяопераційний біль; діти; лікування болю