

Hypocalcemia: A possible risk factor for anastomotic leak in digestive surgery

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Abstract. Anastomotic leaks (ALs) remain the most severe complication in digestive surgery, as well as the most consumptive in terms of human and financial resources. There is an abundance of international research which has focused on identifying and correcting risk factors, and on individualized surgical management as well. The most frequent risk factors are male sex, obesity, diabetes, advanced malignant disease, ASA score, perioperative blood loss or perioperative transfusion, long operation time, emergency operation and altered nutritional status. The aim of the present study was to measure the preoperative serum calcium level and to find a possible correlation between calcium levels and the risk of AL occurrence. A retrospective analysis of medical records for 122 patients who underwent surgical gut resection

with anastomosis for different pathologies was carried out. Preoperative serum calcium level and the occurrence of AL was noted. The results revealed that the average value of total blood calcium was 8.78 mg/dl, without a significant difference in sex groups. Hypocalcemia was identified in 44 patients (36.1%). AL was identified in 8 patients (6.6%), with a statistically insignificant difference between male and female patients. The average value of blood calcium in the AL patient group was 8.07 mg/dl, while in patients without AL the average value was 8.83 mg/dl. Hypocalcemia, defined as a serum calcium level below 8.5 mg/dl, was observed in 7 of the 8 patients presenting with AL (87.5%) and 37 patients who did not present with AL (32.5%), a significant difference with which to consider and include hypocalcemia in the group of risk factors for AL ($P=0.001$). In conclusion, preoperative low serum calcium level can represent a risk factor for AL in digestive surgery.

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Introduction

Gastrointestinal resections represent a common procedure in general surgery, especially in the context of increasing incidence of oncological pathologies (1).

Ideally, resection of a digestive segment is followed by an anastomosis in the same surgical procedure. However, this is not always possible due to local and general conditions of the patient. It is the responsibility of the surgical team to decide whether an anastomosis is both safe and is the best option for

the patient, or whether they have to consider another procedure (performing a temporary or permanent stoma).

The essential rules for a reliable anastomosis are: Ensuring good exposure and adequate blood flow in the anastomosis and lack of tension (2,3). The assurance of these factors have failed to significantly decrease the incidence of postoperative anastomotic leaks (ALs) (4). Thus, it is hypothesized that these conditions are necessary but not sufficient. Additional factors can negatively influence the natural healing process, resulting in the occurrence of AL. Identifying these negative factors and those patients who are at risk can alter the therapeutic strategy for moving patients to a safer area, reduce the hospital stay, morbidity and mortality. To date, to the best of our knowledge, no studies exist which have investigated the possible effects of serum calcium level on AL.

Thus, the aim of the study was to assess the serum calcium level (Ca) in patients with AL and to establish whether there is a correlation between calcium and the risk of AL occurrence, defined as a defect of the intestinal wall at the anastomotic site and leak of luminal contents in the extraluminal space.

Patients and methods

Patients. A retrospective analysis of the medical records of a consecutive 122 patients who underwent surgical resection of the gut for different pathologies, between January 2015 and December 2019, at the 1st Department of General Surgery of the University Emergency Hospital, Bucharest, was carried out. This study was approved by Ethics Committee of the Emergency University Hospital of Bucharest (Romania). Written informed consent was obtained from the patients prior to publication

The inclusion criteria included: i) both sexes, ii) age ≥ 18 years, iii) benign or malignant pathologies, iv) surgical procedure with at least one intestinal anastomosis, and v) known preoperative serum calcium level.

Conversely, the exclusion criteria were: i) age < 18 years, ii) unknown preoperative serum calcium level, and iii) surgical procedure without anastomosis or with protective stoma.

For each patient, data such as sex, age, place of origin, hospitalization regime, type of pathology, type and technique of the performed anastomosis, length of hospitalization, associated pathologies, occurrence of AL and complications and various laboratory data (hemoglobin, protein status, serum calcium) were extracted from the records.

The diagnosis of AL was established by clinical examination, radiological examination, endoscopy or reoperation.

Statistical analysis. Collected data were processed using statistical analysis programs Microsoft Excel 2016 and Epi Info™ 3.5.3 (<https://www.cdc.gov/epiinfo/index.html>; Centers for Disease Control and Prevention; CDC). We used a 95% confidence interval (CI), and the standard deviation (SD) was calculated and differences were considered statistically significant at a probability value of $P < 0.05$.

Results and discussion

The characteristics of study group are described in Table I. Out of 122 patients, 66 were males (54.1%) and 56 were

Table I. Description of the study group (N=122).

Characteristics	n	%	95% CI
Sex			
Male	66	54.1	44.8-63.2
Female	56	45.9	36.8-55.2
Place of origin			
Urban	87	71.3	62.4-79.1
Rural	35	28.7	20.9-37.6
Age group (years)			
25-34	1	0.8	0.0-4.5
35-44	4	3.3	0.9-8.2
45-54	14	11.5	6.4-18.5
55-64	23	18.9	12.3-26.9
65-74	40	32.8	24.6-41.9
75-84	37	30.3	22.3-39.3
> 85	3	2.5	0.5-0.7
Type of pathology			
Malignant	111	91.0	84.4-95.4
Benign	11	9.0	4.6-15.6
Admission			
Scheduled	110	90.2	83.4-94.8
Emergency	12	9.8	5.2-16.6
Anastomotic leak			
Yes	8	6.6	2.9-12.5
No	114	93.4	87.5-97.1

females (45.9%), with a mean age 65.5 years (SD=11.04 years). More than 2/3 of the patients came from urban areas (87 cases, 71.3%). Malignant pathology was the most common type of pathology (111 cases, 91.0%).

AL was identified in 8 patients (6.6%; 95% CI: 2.9-12.5); 4 male cases (6.1%, 95% CI: 1.7-14.8) and 4 female cases (7.1%; 95% CI: 2.0-17.3), with statistically insignificant difference ($P=0.06$). In 6 cases, AL occurred in patients with oncological pathology (5.4%; 95% CI: 2.0-11.4).

Death was recorded in 3 cases (2.5%; 95% CI: 0.-7.1), of which 2 cases were among patients with AL (2.5%; 95% CI: 34.9-96.8) and one case among patients without AL (0.9%; 95% CI: 0.0-4.8).

The average hospitalization period was 16.2 days, with values between 7 and 72 days (SD=9.08 days). The average duration of postoperative hospitalization was 10.4 days, with values between 5 and 65 days (SD=6.98 days). The whole duration of hospitalization and the postoperative hospitalization were higher in patients with AL (Table II).

Most anastomoses were performed manually (93 cases, 76.2%; 95% CI: 67.7-83.5), single-layer (92 cases, 98.9%; 95% CI: 94.2-100), continuous (88 cases, 94.6%; 95% CI: 87.9-98.2), using polypropylene monofilament (91 cases, 97.8%; 95% CI: 3.9-31.7).

AL occurred in 4.3% of cases after hand-sewn bowel anastomosis (4 cases; 95% CI: 1.2-10.6) and in 13.8% of cases that underwent mechanical anastomosis (4 cases; 95% CI: 3.9-31.7).

Table II. Period of hospitalization in patients with and without AL.

Group	Total hospitalization (days)					Postoperative hospitalization (days)				
	Min	Max	Avg	SD	P-value	Min	Max	Avg	SD	P-value
Patients with AL	12	72	34.4	18.5	<0.01	9	65	29.1	16.9	<0.01
Patients without AL	7	50	14.9	6.5		5	18	9.1	2.8	

AL, anastomotic leak.

Table III. Blood calcium levels in the different categories of patients.

Category	Serum calcium level				
	Average (mg/dl)	Minimum (mg/dl)	Maximum (mg/dl)	SD	P-value
Sex					
Male	8.77	6.90	10.80	0.85	0.89
Female	8.79	7.00	10.40	0.85	
Place of origin					
Urban	8.71	6.90	10.80	0.84	0.14
Rural	8.96	7.00	10.47	0.86	
Type of pathology					
Malignant	8.80	6.90	10.80	0.84	0.30
Benign	8.52	7.00	9.72	0.97	
Age group (years)					
25-34	9.20	9.20	9.20	0.00	>0.05
35-44	9.09	7.70	10.01	1.10	
45-54	9.09	7.10	10.80	0.97	
55-64	8.75	7.20	10.10	0.78	
65-74	8.60	7.30	9.80	0.70	
75-84	8.84	6.90	10.40	0.97	
>85	8.72	8.20	9.45	0.65	
Admission					
Scheduled	8.84	6.90	10.80	0.84	0.01
Emergency	8.21	7.00	9.70	0.69	
AL					
Yes	8.07	6.90	9.40	0.82	0.01
No	8.83	7.00	10.8	0.83	
Anastomosis type					
Hand-sewn	8.71	6.90	10.8	0.85	0.09
Mechanical	9.01	7.10	10.40	0.79	

AL, anastomotic leak.

Preoperative total blood calcium was determined and the relationship between its level and the occurrence of AL was studied. Normal values and reference ranges may vary among laboratories and we considered a value between 8.5 and 10.5 mg/dl as normal. In this study hypercalcemia was defined as a blood calcium level >10.5 mg/dl and was recorded in one patient only (0.8%; 95% CI: 0.0-4.5). We considered hypocalcemia as a level of total blood calcium level <8.5 mg/dl, which was identified in 44 patients (36.1%; 95% CI: 27.6-45.3).

The average value of total blood calcium was 8.78 mg/dl (values between 6.90 and 10.8 mg/dl), without a significant difference between sex groups (8.79 mg/dl in women and 8.77 mg/dl in men) (Table III).

No statistically significant differences were found between the presence of malignant pathology and the presence of hypocalcemia (P=0.30). We noted that the mean value of serum calcium was inversely proportional to age, describing a downward curve within the normal limits (Table III).

Table IV. Blood calcium levels in patients with and without CKD.

Group	Blood calcium levels (mg/dl)				P-value
	Min	Max	Avg	SD	
Patients with CKD	7.30	9.50	8.39	0.79	0.15
Patients without CKD	6.90	10.80	8.81	0.85	

The mean calcium level in patients with AL was 8.07 mg/dl (6.90-9.40 mg/dl, SD=0.82), while in patients without fistulous complication the mean value was 8.83 mg/dl (7.00-10.8 mg/dl, SD=0.83) (Table III).

Hypocalcemia was observed in 7 of 8 patients with AL (87.5%; 95% CI: 47.3-99.7) and in 37 of 114 patients who did not have AL (32.5%; 95% CI: 24.0-41.9), a statistically significant difference (P=0.001).

Analyzing chronic kidney disease (CKD) as a possible determinant of hypocalcemia, we found that in patients with this pathological condition, the mean value of serum calcemia was 8.39 mg/dl (7.30-9.50 mg/dl, SD=0.79 mg/dl). In patients without CKD, the mean value was 8.81 mg/dl (6.90-10.80 mg/dl, SD=0.85 mg/dl) (Table IV). In this study the incidence of CKD was 7.4% (9 patients; 95% CI: 3.4-13.5). We found also that hypocalcemia was present in 5 of 9 patients with CKD (55.6%; 95% CI: 21.2-86.3), and in 4 patients (44.4%; 95% CI: 13.7-78.8) the serum calcium was normal.

In only one of patients with CKD was the occurrence of AL (11.1%; 95% CI: 0.3-48.2%) noted. We were unable to demonstrate that CKD is a risk condition for AL (P=0.20), but knowing the effect of this condition on mineral metabolism (induced hypocalcemia, hyperphosphatemia) (5) and the negative effect (especially of hyperphosphatemia) on cardiovascular condition (6-9) other studies with a greater number of patients with CKD are needed.

In 6 of the patients with hypocalcemia, wound complications such as defective scarring, suppuration, marginal necrosis, were observed.

Anastomotic leakage (AL) remains one of the most severe complications in digestive surgery, consuming extensive human and financial resources, with a major psychological impact on the surgical team and the patient. In addition, AL is a risk factor for postoperative death and a negative determinant of morbidity.

Despite the continuous knowledge based on a large number of studies which have focused on risk factors for AL, and the invention of a variety of accessible devices for mechanical anastomosis, especially in hard-to-reach locations, the occurrence of AL has remained at almost constant values for decades, between 2.9 and 15.3% (10).

Correctly performed surgical technique, a good vascularization of margins and tension-free sutures are absolutely necessary conditions but not sufficient in order to obtain a safe anastomosis.

Studies have identified several risk factors that include (but are not limited to): Advanced age (>65 years), ASA class III and IV (11), male sex (12,13), obesity, smoking, alcohol consumption (14,15), history of chemo- or radiotherapy

(especially for endopelvic anastomosis) (16), emergency surgery (17), intraoperative blood loss (18,19), the necessity of blood transfusion (20-22), and also the oxidative stress and the use of several compounds able to modulate oxidative stress (23). Therefore, the complex process of healing of the intestinal anastomosis is directly related to oxidative stress, where calcium plays a pivotal role (24-26).

There are no final conclusions regarding the benefits of preoperative mechanical or chemical bowel preparation. For complicated wounds (dehiscent, suppurative), a gel with polyhexanidine was used for 7 days, due to its antiseptic properties (27). After this period, silver dressing was applied in order to accelerate healing (28,29).

Knowing the importance and involvement of calcium in the healing stages, we focused our attention on the correlation between preoperative values of blood calcium and the risk of developing AL.

The classic phases of wound healing (inflammation, proliferation and remodeling), as described in skin (30), exist in all types of tissue. However, there are significant differences between skin and gastrointestinal healing (31). These differences relate to collagen and collagenase activity, wound strength and wound environment (32). Although it is known that the healing process is more rapid in the gastrointestinal tract than in the skin, the full mechanism of healing and the role of different factors have not been completely elucidated (31).

The process of epithelization in gut mucosa is the result of a perfect equilibrium of migration, proliferation and differentiation of the epithelial cells at the margins of wound (33), with the involvement of different classes of endogenous factors: Cytokines, growth factors, chemokines, prostaglandins (34) or exogenous compounds, able to modulate inflammation and oxidative stress. It was shown that after surgical trauma, an increase in oxidative stress appears and in response, antioxidant enzymes are released; glutathione peroxidase was found to be increased in parallel to glutathione decrease inside the scar tissue. Studies have shown the role of antioxidant systems which are essential for the healing process (35-37).

Until recently, very little was known regarding how damaged tissue activates and attracts the first white blood cells to the wound. Researchers from the University of Bristol's School of Biochemistry in collaboration with a team from the University of Bath, have shown that the very first trigger in this process is a flash of calcium which spreads like a wave back from the wound edge through the gap junctions that connect all the cells. This flash of calcium signal goes on to activate an enzyme known as DUOX (dual oxidase), which synthesizes hydrogen peroxide, therefore attracting white blood cells into the wound. The wound-induced calcium flash represents the earliest identified signal following wounding and might therefore orchestrate the rapid recruitment of immune cells. Studies have shown that the inhibition of that flash of calcium reduces H₂O₂ production and negatively affects white blood cell migration (38).

A study on cultured cells in the presence of medium containing different calcium concentrations (from 0.1 to 3.5 mM Ca²⁺) reports that a concentration of 3.5 mM of CaCl₂ increases metabolic activity in *in vitro* wound closure, matrix metalloproteinase activity, collagen synthesis and cytokine expression, reducing cell contraction capacity (39).

Extracellular Ca^{2+} is a crucial regulator of epidermal homeostasis and its receptor (Ca^{2+} -sensing receptor) conveys the extracellular Ca^{2+} signals to promote keratinocyte adhesion, differentiation and survival via activation of intracellular Ca^{2+} and E-cadherin-mediated signaling (40). The same authors confirmed that ablation of Ca^{2+} -sensing receptor (CaSR) delays wound repair, as well as the deletion of the vitamin D receptor (VDR) due to the perturbation of a large number of pathways involved in wound healing. CaSR also activates the Rho GTPase-mediated signaling in order to facilitate actin-cytoskeleton remodeling and the formation of E-cadherin/catenin adherens junction, which play a mandatory role in transducing the outside-in signals, by activating and integrating various intracellular signaling cascades. To the best of our knowledge, we have not found a similar study on intestinal tissue.

Oda *et al* demonstrated that restricting dietary calcium (or deleting CaSR) affected wound healing in mice (41,42).

Evolution of the healing process in the gastrointestinal tract is anatomically hidden from inspection. Although similarities with this process in the cutaneous tissue exist, gastrointestinal anastomotic healing should be considered a separate entity.

The full implication of calcium in wound healing in general and in anastomosis healing in particular requires elucidation. The mentioned observations suggest its role not only in hemostasis, but in repairing and regenerating processes as well. The majority of studies regarding wound healing describe this phenomenon in skin tissue. Due to the importance of a competent, well healed anastomotic suture in surgical practice, more attention may be required in regards to this site.

The bidirectional effect of arterial hypertension (AH) and CKD is known (43). In addition, other studies show the causal relationship between diabetes and CKD (43-47), as well as increased cardiovascular and death risk in patients with CKD (5). Other studies are needed to investigate the correlations among AH, diabetes, CKD, other hypocalcemic pathological conditions (hypoparathyroidism, malabsorption) and AL.

In conclusion, the healing process starts immediately after injury and depends on chemical mediators. Accepting the importance of calcium on different stages of healing determined us to investigate the correlation between blood calcium level and the incidence of AL in patients with hypocalcemia. This study found that: i) there was no significant difference between male and female patients regarding AL incidence; ii) emergency surgery was a risk factor for AL, compared to scheduled surgery; iii) no statistically significant differences were found between benign or malignant pathologies and hypocalcemia; iv) preoperative hypocalcemia was identified in most of the cases of patients with AL; v) preoperative serum calcium level may be used to identify patients at risk for AL. These data suggest that hypocalcemia can represent another risk factor for AL, although the complete mechanism is unclear and more studies are required.

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Availability of data and materials

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

Authors' contributions

CB, DS, DAM, DGB, PCC, OS, AT, ME, DM, DEG, TFG and DCB designed the study, performed the literature search and selected the included studies. CB, DS and DAM performed statistical analysis and interpretation of the results. DGB and ME wrote the paper. DV and AI critically revised the manuscript and analyzed the results for accuracy. All authors read and approved the final version of manuscript. The contributions of all the authors to this study are greatly valued and appreciated.

Ethics approval and consent to participate

This study was approved by Ethics Committee of the Emergency University Hospital of Bucharest (Romania) (approval no. 50706/08.10.2020). Written informed consent was obtained from the patients prior to publication.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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