

## Цитоморфологічне дослідження клітинного складу перипанкреатичних рідинних скупчень в оцінюванні тяжкості клінічного перебігу гострого ускладненого панкреатиту

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## Cyto-morphological investigation of cellular content of peripancreatic liquid accumulations in estimation of the clinical course severity in an acute complicated pancreatitis

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### Реферат

**Мета.** Вивчити характер та особливості цитоморфологічних змін клітинного матеріалу осаду перипанкреатичних рідинних скупчень (РС) як критерію об'єктивної оцінки тяжкості клінічного перебігу гострого ускладненого панкреатиту (ГУП).

**Матеріали і методи.** Проаналізовано результати цитоморфологічного дослідження осаду перипанкреатичних РС, добутого під час виконання інтервенційної ультрасонографії – 51 (88%) спостереження та відеолапароскопії – 7 (12%) спостережень у 58 хворих із ГУП.

**Результати.** У разі внутрішньочеревних РС (n=37) у 10 (27%) спостереженнях виявлено домінування проліферуючих клітин мезотелію з їх поліморфізм, у 15 (41%) – дегенеративно-дистрофічні зміни у вигляді вип'ячувань цитоплазми, появу великих «перснеподібних» клітин, у 12 (32%) – атипично змінени клітини мезотелію з проявами анізоцитозу та анізонуклеозу, а також цитоморфологічні зміни, подібні до неопластичного процесу. У разі позаочеревинної локалізації РС (n=21) фіксували істотніші зміни ядер гранулоцитів, зокрема, у 9 (43%) спостереженнях їх структура мала незворотні порушення у вигляді явищ каріопікнозу, каріорексису та каріолізису. Глибина структурних змін клітин мезотеліоцитів та нейтрофільних гранулоцитів залежала від ступеня тяжкості ГУП.

**Висновки.** Визначення глибини цитоморфологічних порушень клітинного складу осаду РС може слугувати об'єктивним критерієм оцінки ступеня тяжкості ГУП.

**Ключові слова:** гострий ускладнений панкреатит; рідинні скупчення; цитологічне дослідження.

### Abstract

**Objective.** To study up a character and peculiarities of cyto-morphological changes in cellular material of the peripancreatic liquid accumulations (LA) as a criterion of objective estimation of the clinical course severity in an acute complicated pancreatitis (ACP).

**Materials and methods.** Results of cyto-morphological investigation of the peripancreatic LA sediment, obtained while doing interventional ultrasonography in 51 (88%) observations and videolaparoscopy – in 7 (12%) observations in 58 patients with an ACP, were analyzed.

**Results.** Of intraabdominal LA (n=37) observations in 10 (27%) of them a domination of proliferating cells of mesothelium with their polymorphism was revealed, while in 15 (41%) – degenerative-dystrophic changes like a bulging of cytoplasm, appearance of large «signet-ring like» cells, in 12 (32%) – atypically changed cells of mesothelium with signs of anizocytosis and anizonucleosis, as well as cyto-morphological changes like in neoplastic process. In retroperitoneal localization of LA (n=21) a more essential changes in the granulocytes nuclei were registered, including, in 9 (43%) observations their structure have got irreversible disorders: karyopycnosis, karyorexis and karyolysis. The depth of structural cellular changes in mesotelocytes and neutrophil granulocytes have depended upon the ACP severity.

**Conclusion.** Determination of depth of the cellular content cytomorphological disorders in the LA sediment may serve an objective criterion in the ACP severity estimation.

**Keywords:** acute complicated pancreatitis; liquid accumulations; cytological investigations.

The updated classification of acute pancreatitis (AP) (Atlanta, 2012) provides an in-depth description of local disease complications and, first of all, pancreatic fluid collections (FC) [1]. Thus, the following FC types distinguished. 1. Acute peripancreatic FC with aseptic contents without signs of pancreas necrosis and parapancreatic fat, which are aseptic (Acute peripancreatic fluid collection – APFC). 2. Acute necrotic FC with different amounts of both fluid and necrotic tissue fragments associated

with the necrosis in pancreas parenchyma and/or parapancreatic tissue (Acute necrotic collection – ANC). 3. Post-necrotic infected pancreatic and/or parapancreatic FC with contents in the form of a fluid component/purulence and necrotic tissue/detritus predominantly infected (Post-necrotic pancreatic/retropancreatic fluid collection – PNFC).

Remarks to this classification indicate that the operational tactical approaches in these complications have their own

characteristics [2,3]. Is it possible to assess FC content in determining the severity of the clinical course of disease?

Purpose studies: to study the character and peculiarities of cytomorphological changes of cellular sediment material of fluid collections (FC) as criterion for objective assessment of the clinical course severity in acute complicated pancreatitis (ACP).

### Materials and methods studies

Cytomorphological study of peripancreatic FC sediment produced during interventional ultrasonography in 51 (88%) and video–laparoscopy in 7 (12%) observations was performed in 58 patients who were under the inpatient treatment in the City Pancreatic Centre at the Academic Department of General Surgery of Danylo Halytsky Lviv National Medical University, with a confirmed ACP diagnosis (based on clinical, laboratory–biochemical, radiological and instrumental methods). The age of hospitalised patients ranged from 22 to 74 years. 36 female (33%), 73 male (67%). The cytological study of pancreatic and peripancreatic FC contents provided for the implementation of two stages – pre–analytical and analytical. The first one included qualified collection and transportation of study material, which was important for further result. The analytical stage provided for a cytological study of native and stained samples. Samples were Pappenheim–stained.

Fluid collections content was transferred into test tube or other, more spacious, clean, dry labware with 5% sodium citrate solution at a rate of 2 – 5 ml per 100 ml of the test material in order to stabilize it and prevent the destruction of cellular elements. With a considerable volume of fluid material, it was centrifuged at 1500 rpm for 5 – 10 minutes, after which supernatant was discharged, and 4–6 preparations were received from the precipitate with drying for 1 hour and subsequent staining. Further study was performed using BIOLAM and MIKMED–5 light microscopes with a magnification of  $\times 40$ ,  $\times 90$ ,  $\times 100$ . Microscopy was applied to both native and stained specimen with small and large microscope magnifications (immersion system). The obtained study results were processed using variation statistics of t–test, Fischer test and  $\chi^2$ .

### Results

The obtained study data provided grounds to state that as a result of the aggressive environment effect of the peripenetrating fluid collections with high levels of activated enzymes

and proinflammatory cytokines on cellular elements resulted in pathological changes of all cellular elements as determined in cytological specimens. However, the most significant and indicative were the changes in FC mesotheliocytes with intra–abdominal location (37 observations), since these cells, being the main structural elements of serous membranes, in particular parietal and visceral peritoneum, were in direct contact with enzymatically active pancreatic exudate.

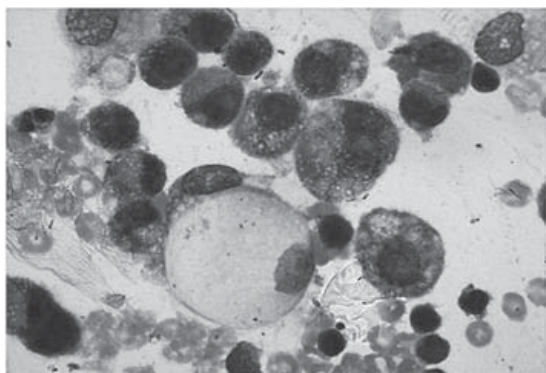
Thus, 10 (27%) studies showed dominance in cytological specimens of proliferating mesothelium, characterized by large mesotheliocytes, nuclei and their polymorphism. 15 (41%) cases showed degenerative–dystrophic changes in both cytoplasm and mesotheliocytes in the form of cytoplasmic protrusions, its vacuolation, appearance of foaminess, sometimes with large castration cells. 12 (32%) studies showed the presence of atypically altered mesothelium cells with anisocytosis and anisonucleosis, as well as cytomorphological changes similar to neoplastic process (*fig. 1, 2*).

Cytomorphological abnormalities of peripancreatic FC content with their localization in retroperitoneal space (21 observations), in the absence of direct contact of the pancreatic fluid with peritoneum, were most significant in neutrophilic granulocytes. Thus, all studies showed changes in these cells cytoplasm in the form of toxic grains and vacuolations both in individual cells and most neutrophil granulocytes, intensive basophilia, decreased specific graininess and appearance of circular colourless spots, which reflected the initial process of degenerative–dystrophic cell changes.

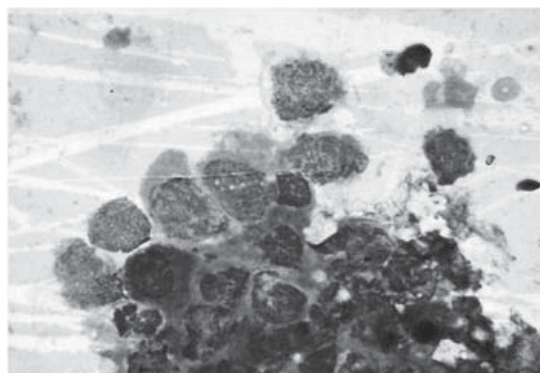
However, more significant changes were observed in granulocyte nuclei. Thus, in 9 (43%) cases, irreversible structural damages were observed characteristic of the gradually–sequential stages of death, namely karyopyknosis, karyoclasia and karyolysis (*fig. 3, 4*).

An analysis was performed of cytomorphological changes in mesotheliocytes and neutrophil granulocytes with intra–abdominal and retroperitoneal FC location depending on the severity of the clinical course of disease according to AP classification criteria (Atlanta, 2012) [1] (*table 1, 2*).

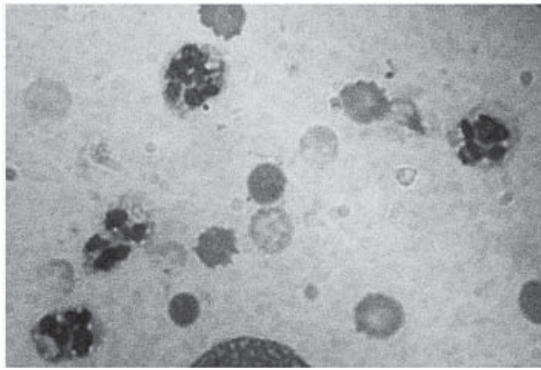
As can be seen from the data presented in tables, in moderately severe ACP, in contrast to the severe clinical course of the disease, the cytological presentation of intra–abdominal FC was characterized by damage to mesotheliocytes structure in the form of their degenerative–dystrophic changes – in 14



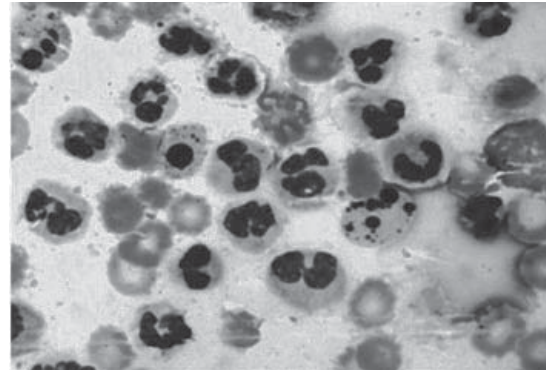
*Fig. 1.*  
*Degenerative–dystrophic changes in cytoplasm. Castration cell.*



*Fig. 2.*  
*Atypically altered mesothelium cells. Anisocytosis, anisonucleosis, polymorphism of cells and nuclei.*



*Fig. 3.*  
*Vacuolation of the neutrophil granulocytes cytoplasm.*



*Fig. 4.*  
*Changes in granulocyte nuclei. Karyopyknosis, karyoclasia and karyolysis.*

Cytomorphological changes in mesotheliocytes	Moderately severe ACP (n=24)		Severe ACP (n=13)	
	abs.	%	abs.	%
Mesotheliocytes proliferation	9	38	1	8
Degenerative-dystrophic changes in cytoplasm and mesothelium nuclei	14	58	1	8
Polymorphism of mesothelium cells and nuclei (cellular atypia)	1	4	11	84
Total ...	24	100	13	100

Cytomorphological changes of neutrophil granulocytes	Moderately severe ACP (n=14)		Severe ACP (n=7)	
	abs.	%	abs.	%
Degenerative-dystrophic changes in cytoplasm	13	93	2	29
Destructive damages of nucleus – karyopyknosis, karyoclasia and karyolysis	1	7	5	71
Total ...	14	100	7	100

(58%) observations vs. 1 (8%) case ( $\chi^2=6.593$ ;  $p=0.01$ ), while the incidence of mesotheliocytes proliferations did not have statistically significant differences – 9 (38%) vs. 1 (8%) of observations ( $\chi^2=2.438$ ;  $p > 0.05$ )

This severe clinical course of the disease was characterized by atypical forms of mesotheliocytes and their nuclei, similar to cells of neoplastic genesis, as observed in 11 (84%) observations vs. 1 (4%) cases ( $\chi^2=21.368$ ;  $p < 0.001$ ).

The peculiarities of neutrophil granulocyte changes in the retroperitoneal peripancreatic FC were that both in moderately severe and severe ACP there were cytomorphological damages of cells cytoplasm in the form of degenerative–dystrophic changes. However, in the severe course of disease, in addition to cytoplasm, cell nuclei were also affected by destructive process in the form of karyopyknosis, karyoclasia and karyolysis – 5 (71%) observations vs. 1 (7%) case ( $\chi^2=10.286$ ;  $p=0.001$ ). Consequently, the depth of structural changes in mesotheliocytes and neutrophil granulocytes depended on ACP severity. The obtained results provided grounds to work out the “Assessment method of acute necrotic pancreatitis severity” and “Prognostication method for clinical course of acute destructive pancreatitis” [4, 5], according to which the evaluation of cytomorphological changes in mesotheliocytes

and neutrophil granulocytes in peripancreatic FC content can serve as a commonly available criterion for determining ACP severity.

### Discussion

An unbiased assessment of ACP severity is the basis for determining treatment strategy and tactics in patients with this disease. However, the existing integral prognostication scales for disease severity as a general intensive care profile (APACHE II, SAPS, MODS, SOFA), and specifically adapted for acute pancreatitis (Ranson, Leere, Blamey, Balthazar, BISAP) are quite cumbersome and inaccessible to practical application [6, 7]. Recently, studies have been widely conducted to identify certain criteria for disease severity, the so-called ACP predictors, such as the emergence of hydrothorax [8], changes in erythron parameters and iron metabolism [9], neutrophils damage system [10]. The proposed method is affordable, cost-effective and fast, which greatly contributes to addressing this issue.

### Conclusions

1. Clinical course of ACP shows cytomorphological changes in peripancreatic FC contents, which are the most significant

and indicative in mesotheliocytes with intra–abdominal FC localization and neutrophil granulocytes with their retroperitoneal arrangement.

2. Assessment of the extent of identified cytomorphological damage in cellular composition of FC sediment can serve as an objective and publicly available criterion of ACP severity.

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