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Diagnostic significance of determining the etiological factor at sepsis in babies by of gas-liquid chromatographic method

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Abstract
The possibility of using the GC method for express and accurate determination of markers of microorganisms in various biological materials with subsequent calculation of the microorganism titer is shown in the work. At the same time, the data on the composition of microorganisms obtained for each patient, when assessing the overall microecological status, allow the doctor to obtain qualitatively new extensive information for the adoption of adequate antimicrobial and general therapy. There is a high correlation between the contents of markers of that microorganisms in various biological objects for sepsis in infants, which shows the determination of the content of markers does not depend on the object in this pathology.

Keywords: sepsis, infants, diagnosis, markers of microorganisms, gas-liquid chromatography

1. Introduction
Sepsis remains an urgent problem of pediatrics, caused by high mortality. To date, the problem of laboratory diagnosis of sepsis has not been resolved. The results of a large number of studies carried out in recent years indicate that the role of various inflammatory and saprophytic aerobic and anaerobic representatives of the intestinal microflora has been increased in the development of purulent-inflammatory diseases, sepsis and other severe infectious diseases [2, 4], which determines the features Inflammatory process developmental.

At the same time, lethality in proper treatment of anaerobic septicemia does not exceed 10%, and in inadequate ore - it can reach 60-80% [3, 6], which is associated with accurate diagnosis of the etiologic factor. However, the methods of classical microbiological diagnosis (blood culture), with the isolation of a pure anaerobic culture are laborious and not always available [3]. Recently, the diagnosis of anaerobic purulent inflammatory processes uses the definition of metabolites of anaerobic bacteria, which are low-molecular compounds and are specific markers, such as volatile fatty acids (VFA), which allow to determine quickly and reliably small amounts of substances of microbial origin including anaerobes in any biological environment of the body and provide reliable results. Integration of the metabolism of humans the being and indigenic anaerobes with the example of volatile fatty acids, which include propionic (propionate), butylate (butyrate), valerian, caproic and some others, all of which are specific for strict anaerobes as the final products of metabolism and are not formed by human cells. The fact of detection of VFA in biological media and tissues is used to diagnose anaerobic infections [4, 6, 8].

In this regard, the use of readily volatile fatty acids as specific markers is relevant from the point of view of typing the causative agent of infection.

The aim of the study was to study the diagnostic significance of the detection of microorganism's markers by gas-liquid chromatographic method in children with sepsis Materials and methods.

2. Material and Methods of investigation
The study of the course of sepsis in 79 children aged from 2 months. Up to 1 year being treated, were in the intensive care unit for purulent-septic patients of the branch of the Children's Surgery of Samarkand. In 56 cases, a septicemia sepsis was diagnosed, and in 23 cases septicpyemia was diagnosed. The comparison group included 20 practically healthy children of similar age.

Gram-positive and gram-negative aerobes were revealed by a classical bacteriological method. Anaerobic infection was revealed by gas-liquid chromatography [1].

The GLC method is based on the determination of the markers of fatty acid microorganisms in the form of their methyl esters: the stationary phase - 15% was ground lestosil on a NAW chromatograph with a particle size of 0.150-0.250 mm, in a glass column measuring 0.04x1.00 m; flow rate of nitrogen gas - 32 ml / min; detector of - flame ionization ratio, nitrogen: hydrogen: air ratio = 1: 1: 10, introduced sample volume - 2-3 µl of hexane extract of methyl esters of fatty acids (from the analyzed sample, the lipid fraction was isolated according to the Folch method, methyl esters of fatty acids were obtained by transesterification Glycerides with methanol in the presence of acetyl chloride [3].

The identification of fatty acids was carried out by the method of "witnesses" and on the basis of the method of structural-group constituents [3, 4], and quantitative analysis by the absolute calibration method [6]. Statistical processing of data was carried out using the package of applied programs Statistica 6.0.

3. Results & Discussion
Taking into account the most widespread point of view concerning the paramount importance of bacteremia in the development of sepsis, a study of blood for sterility was carried

out in all examined patients immediately on admission to the hospital. The data on the spectrum of the isolated from the clinical material (blood, inculcation from wounds, drainage, fistula, from the pharynx, inculcation urine, from feces) microorganisms are given in table 1.

Table 1: The frequency of allocation of different groups of microorganisms from the clinical material, % (n=79)

Clinical material	Growth of culture	Gram (-) sticks	Gram (+) sticks	Fungi of genus Candida	Gram (-) Gram (+) mixed
Blood	43,2	48.2	36,2	5,6	-
Inculcation from wounds, drainage, fistula	91,5	61,8	30,2	7,9	-
Inculcation from the mouth	78,1	36,6	41,3	5,3	16,6
Urine culture	26,5	45,1	33,3	17,6	3,9
Inculcation feces	94.7	45,0	34,1	6,1	14,8

As can be seen from the table, the detection of microorganisms from the blood was noted only in 43.2% of cases. In the structure of blood cultures, gram-positive bacteria were at the level of 36.2%, with the prevalence of staphylococcus aureus - 63.3%, while the growth of epidermal staphylococcus cultures was noted in 33.3% of cases, and streptococcus in 3.4% of cases. Gram-negative rods in the blood culture were detected in 48.2% of cases, of which Proteus - in 37.5%, E. coli - in 52.5%, Pseudomonas aeruginosa - in 5%, Klebsiella - in 5% of cases. Growth of fungi of the genus Candida in hemoculture was noted in 5.6% of the examined patients - amounted to 5.6%.

Thus, the results of the analysis of the bacteriological spectrum of hemocultures of aerobic flora indicate a competing role of representatives of gram-negative and gram-positive flora in the etiological structure of sepsis in infants.

The study of anaerobic infection by GLC method revealed that the most significant causative agents for sepsis in infants were clostridia, peptostreptococcus (gram-positive anaerobes), fusobacterial (gram-negative anaerobes) (table 2).

Table 2: The content of markers of microorganisms in the blood of children

	Microorganism	Marker	Sepsis
1	Peptostreptococcus anaerobus	Isolauric acid iC12	$Y=8,23*10^{5}*x$
2	Propionibacterium	Isopentadecanoic acid i15	$Y=5,36*10^{5}*x$
3	Fuzobacterium	3 hydroxy-palmitic acid	$Y=7,14*10^{5}*x$
4	Clostridium ramosum	9.10 tetradecenoic acid 15: 1Δ9	$Y=8,16*10^{5}*x$
5	Enterococcus faecalis	Cyclononadecanoic acid (19cyc)	$Y=10,22*10^{5}*x$
6	Lactobacillus	1-Methylene octadecanoic acid (C19cyc)	$Y=5,73*10^{5}*x$
		Heptadecane aldehyde (7a)	$Y=11,40*10^{5}*x$
		Cyclononadecane aldehyde	$Y=7,52*10^{5}*x$
7	Bifidobacterium	Isooctadecanoic (i18)	$Y=8,33*10^{5}*x$
		Тетрадекановая кислота (14a)	$Y=10,15*10^{5}*x$
		Octadecene aldehyde	$Y=6,12*10^{5}*x$

Note: * Y - the content of the marker in the microorganism;
X- Is the height of the peak in the chromatogram, mm?

Normally, the VFA does not enter the systemic circulation, the utilization of these acids occurs elsewhere in the intestine, they are of exclusively microbial origin, since they are products of anaerobic bacteria and do not form in eukaryotic cells; they serve as a substrate for the intestinal epithelium, ensuring the reliability of the intestinal barrier. At the same time, according to Beloborodova N.V. [8] in pathological integration, these anaerobic metabolites can come from the foci directly to the human internal environment, suppressing immunoreactivity, promoting the development of immunoparality and an unfavorable outcome. During the study of sepsis in children, we studied the content of volatile fatty acids in various biological media (blood, wound exudate and feces), the correlation of the determination of the content of microorganism's markers in various biological objects in children with sepsis was examined. The results are shown in table 3.

Table 3: Comparative content of microbial markers in biological fluids for sepsis in infants.

N	Kind of microorganism	The biological fluid	Correlation coefficient of markers in biological fluids		
			Blood	Wound exudate	Filtrate of faeces
1	Isolauric acid iC12	Blood	1,000	0, 989	0, 926
		Wound exudate	0,989	1,000	0, 945
		Filtrate of faeces	0,926	0,945	1,000
2	Isopentadecanoic acid i15	Blood	1,000	0, 973	0, 944
		Wound exudate	0,973	1,000	0, 978
		Filtrate of faeces	0,944	0,978	1,000
3	3-Hydroxy-Palmitic Acid	Blood	1,000	0, 982	0, 956
		Wound exudate	0,982	1,000	0, 933
		Filtrate of faeces	0,956	0,933	1,000

4	9,10-tetradecenoic acid 15: 1Δ9	Blood	1,000	0, 966	0, 975
		Wound exudate	0,966	1,000	0, 964
		Filtrate of faeces	0,975	0,964	1,000
5	Cyclononadecanoic acid (19cyc)	Blood	1,000	0, 971	0, 992
		Wound exudate	0,971	1,000	0, 948
		Filtrate of faeces	0,992	0,948	1,000
6	1-Methylene octadecanoic acid (C19cyc)	Blood	1,000	0, 984	0, 969
		Wound exudate	0,984	1,000	0, 980
		Filtrate of faeces	0,969	0,980	1,000
7	Isooctadecanoic (i18)	Blood	0,969	0,980	1,000
		Wound exudate	1,000	0, 991	0, 958
		Filtrate of faeces	0,991	1,000	0, 978

It should be noted that in sepsis in infants, the correlation coefficient significance for the content of FLV from different biological media is greater than 0.9, that is, the results are correlated.

In addition, a direct relationship between the severity of the purulent-inflammatory process and the concentration of bacterial markers in biological fluids was revealed.

Results should be the major findings of your experiment. You have to compare the results with previous studies done in same.

4. Conclusion

1. The possibility of using the GC method for express and accurate determination of markers of microorganisms in various biological materials with subsequent calculation of the microorganism titer is shown. At the same time, the data on the composition of microorganisms obtained for each patient, when assessing the overall microecological status, allow the doctor to obtain qualitatively new extensive information for the adoption of adequate antimicrobial and general therapy.
2. There is a high correlation between the contents of markers of that microorganisms in various biological objects for sepsis in infants, which shows the determination of the content of markers does not depend on the object in this pathology.

5. References

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