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Granulocyte Elastase as a Sensitive Diagnostic Parameter of Silent Male Genital Tract Inflammation*

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Summary: Elastase, a specific inflammatory parameter of polymorphonuclear (PMN) granulocytes, was quantified with a sensitive enzyme immunoassay in the ejaculates of 188 patients consulting the andrological outpatient service. Correlations of the elastase concentrations to other parameters used up to now for the diagnosis of silent male genital tract inflammation were statistically evaluated by the CHI^2-test. A correlation was found neither to the percentage of morphologically intact spermatozoa in the differential spermiocytogram and the total number of spermatozoa nor to the pH-value and viscosity of the ejaculate. However, release of elastase into the ejaculates was clearly associated with the occurrence of bacteria in native and stained smears or with the numbers of round cells present. Moreover, leukocyte counts in stained smears as well as an inflammation coefficient were highly significantly correlated to elastase concentrations. Obviously, quantification of granulocyte elastase in seminal plasma enables a rapid diagnosis of silent male genital tract inflammation, since even a single determination gives a reliable criterion and sequential determinations may allow the control of the course of the disease during therapy.

Granulozytäre Elastase als sensitiver diagnostischer Parameter beim stillen Samenwegsinfekt

Zusammenfassung: In den Ejakulaten von 188 Patienten der andrologischen Sprechstunde wurde die aus polymorphkernigen (PMN) Granulozyten freigesetzte Elastase im ng-Bereich mittels eines sensitiven Enzymimmunoassays exakt bestimmt. Dieser erst seit kurzem nachweisbare Entzündungsparameter wurde im Vergleich mit den bisher zur Diagnostik eines stillen Samenwegsinfekts verwendeten Spermiogrammbefunden einer statistischen Analyse (CHI^2-Test) unterzogen. Für die Menge an Elastase war kein signifikanter Zusammenhang mit dem Prozentsatz an morphologisch unauffälligen Spermatozoen, der Gesamtspermatozoenzahl und dem pH-Wert bzw. der Viskosität des Ejakulats nachzuweisen. Dagegen ergab sich eine signifikante Korrelation zum Auftreten von Bakterien im Nativ- oder im gefärbten Ausstrichpräparat bzw. zur Anzahl der Rundzellen. Hochsignifikant war der Bezug zur Leukozytenzahl im gefärbten Ausstrich und zu einem Entzündungskoeffizienten. Im Vergleich zu den bisher verwendeten Verfahren stellt die Quantifizierung der Granulozytenelastase im Ejakulat eine sehr sensitive und selektive Methode zur Abklä-

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rung des Verdachts auf einen stillen Samenwegsinfekt dar und ermöglicht zudem eine weitere Verlaufskontrolle der Erkrankung.

Introduction

Since a few years great interest has arisen in silent male genital tract inflammation as a possible cause of male infertility.

Male reproductive organs are attacked by classic venereal pathogenic organisms as well as by non-venereal bacteria, viruses and microorganisms such as mycoplasmata and chlamydia. Some of these infections are supposed to proceed asymptomatically for years; often they are accidentally detected by analyzing the ejaculate, if the patient visits the andrology unit because of childlessness. Yet, the great number of parameters used up to now for diagnosis clearly indicates the difficulties in confirming silent inflammations of the male efferent duct system.

Elastase, one of the major lysosomal proteinases of polymorphonuclear (PMN) granulocytes, turned out to be a crucial inflammatory mediator if released extracellularly. This enzyme is, therefore, especially suitable as an approved biochemical indicator of inflammation (Fritz et al. - 1984).

Recently determination of granulocyte elastase in body fluids such as seminal plasma by an enzyme-linked immunosorbent assay has made possible a clear discrimination of inflammatory from non-inflammatory processes (Jochum et al. - 1985).

So far, various spermiogram parameters have been used for diagnosis of silent male genital tract inflammation: elevated pH-values of the semen; changes in colour, viscosity and "Spinnbarkeit" of the semen; increased number of round cells and decreased number of spermatozoa; occurrence of epithelial cells, bacteria, trichomonades and erythrocytes; enlarged leukocyte counts; impaired qualitative and quantitative sperm motility; and positive bacterial culture (Eliasson - 1975; Nikkanen et al. - 1978).

Although these parameters in total support the diagnosis of the silent seminal tract infection, its prediction by a single factor is limited due to various sources of error (Mardh and Colleen - 1975; Comhaire et al. - 1980; Riedel and Semm - 1980).

In this study, granulocyte elastase was quantified in ejaculates in addition to the well-known spermiogram parameters. Moreover, an inflammation coefficient, which includes the coincident occurrence of different indications to inflammatory reactions similar to those applied already by Comhaire et al. (1980) for diagnosis of chronic adnexitis, was used as a further criterion to identify an inflammatory process. The correlations of the amount of the granulocyte elastase to the inflammation coefficient on the one hand and to some of the before-mentioned sperm parameters on the other hand were statistically evaluated.

Materials and Methods

Semen analyses: 188 ejaculates of 18 to 59 years old patients (mean: 32.9 years) consulting the andrological outpatient service were studied (Schirren - 1982). The native samples were investigated concerning ejaculate volume, pH-value, viscosity, sperm motility, total number of spermatozoa, round cells, erythrocytes and epithelial cells, bacteria and trichomonades. Normal and pathological spermatozoa were differentiated. In stained smears cell type and number of leukocytes as well as erythrocytes and bacteria were evaluated. If necessary, typifying of microorganisms was performed growing them in special culture media.

Quantification of granulocyte elastase: Granulocyte elastase in cell-free seminal plasma was determined by the method of Neumann and Jochum (1984) using an enzyme-linked immunosorbent assay (meanwhile commercially available from E. Merck, Darmstadt). Due to the relatively rapid reaction of extracellularly liberated elastase (E) with its major inhibitor, the α_1-proteinase inhibitor (α_1PI), the enzyme can be detected in body fluids normally only in inactive , complexed form (E-α_1PI).

The principle of the assay is as follows: a) incubation of standards (i.e. the complex produced in vitro) or seminal plasma samples for 1 h in polystyrene tubes coated with sheep antibodies to human granulocyte elastase, b) washing, c) incubation of the tubes containing the fixed complex (E-α_1PI) for 1 h with alkaline phosphatase-labelled rabbit antibodies to α_1-proteinase inhibitor, d) washing, and e) determination of the solid phase-fixed alkaline phosphatase activity with p-nitrophenylphosphate as the substrate (incubation time: 45 min). The change in absorbance measurable at 405 nm is linearly correlated with the concentration of complexed elastase. The amount of elastase in the seminal plasma sample is calculated from the standard curve (note: concentrations in ng/ml are given for the amount of complexed elastase only!).

To get sufficient numbers in different classes for statistical evaluation, three groups were established due to the results of recent studies (Schiessler - 1984): elastase values below 250 ng/ml were classified as 'no inflammation' and values from 250 ng/ml to 999 ng/ml as 'moderate inflammation'; concentrations of more than 1000 ng/ml implied an acute inflammation.

The Cyturtest (Boehringer, Mannheim) recommended for determination of granulocyte-derived unspecific esterase activity in urine was used as an additional screening test.

Inflammation coefficient: For statistical comparison we established a coefficient of inflammation which is based on the parameters used up to now. Thereto, we further developed the diagnostic inflammation test of Comhaire et al. (1980) as follows: The inflammation coefficient is increased by one point each, if

1. more than 10 round cells per microscopic high power field (x 400) are found in the native semen,
2. bacteria or trichomonades are seen in native semen or in stained smears,
3. more than 2 leukocytes per microscopic high power field are found in semen smears,
4. the bacteriological culture shows positive results.

Statistical evaluation: The statistical evaluation between the elastase concentration in semen and the other indicators of inflammatory processes was performed by the CHI^2-test; the probability of error (alpha) is given in percent. Alpha-values lower than 0.1% are considered as highly significant, values from 0.1% to 5% as significant (Pabst - 1985).

Results

Parameters without relevance to granulocyte elastase. The following list is arranged according to the probability of the zero hypothesis = probability of error, i.e., the higher the number of α (%) the lower is the correlation to granulocyte elastase. Parameters with $> 5\%$ have been supposed to show no significant correlation to the amount of elastase in seminal plasma.

α	Parameters
89%	morphologically intact spermatozoa in the differential spermiocytogram
50%	total number of spermatozoa per ejaculate
26%	pH-value of the ejaculate
11%	viscosity of the ejaculate

Table 1
Granulocyte elastase versus microorganisms in native preparations and stained smears

			Concentration range of granulocyte elastase (ng/ml) 0–249		250–999		1000–20000	
		n	n	%	n		n	%
native preparations	no bacteria	126	73	82.0	35		18	71.6
	bacteria or trichomonades	37	16	18.0	8		13	28.4
		163	89	100.0		74		100.0
stained smears	no bacteria	147	86	96.6	38		23	82.4
	bacteria	16	3	3.4	5		8	17.6
		163	89	100.0		74		100.0

Table 2
Granulocyte elastase versus round cells or leukocytes per microscopic high power field (x 400)

			Concentration range of granulocyte elastase (ng/ml) 0–249		250–999		1000–20000	
		n	n	%	n		n	%
round cells	0– 5	57	38	42.7	13		6	25.6
	6–10	56	35	39.3	14		7	28.4
	11–15	27	6	6.7	11		10	28.4
	> 15	23	10	11.3	5		8	17.6
		163	89	100.0		74		100.0
Leukocytes pathologic	no	61	44	49.4	12		5	23.0
	1–2	57	28	31.5	21		8	39.2
	3–5	29	12	13.5	7		10	23.0
	> 5	16	5	5.6	3		8	14.8
		163	89	100.0		74		100.0

Parameters with significant correlation to granulocyte elastase.

Bacteria in the native preparation and stained smears: For this parameter 163 ejaculates were investigated. In 126 native samples (77.3%) no bacterial contamination could be found, whereas 37 samples (22.7%) showed bacteria or trichomonades. The numbers of ejaculates in each of the 3 classes arranged according to the amount of granulocyte elastase (0–249 ng/ml, n = 89; 250–999 ng/ml, n = 43; 1000–20000 ng/ml, n = 31) are given in Table 1. Although at least 18% of ejaculates with low elastase concentrations but only 28.4% of specimens with elastase amounts higher than 250 ng/ml contained bacteria and trichomonades, the statistical evaluation demonstrated a moderate but clear correlation between the quantity of elastase and the occurrence of pathogenic microorganisms in native preparations (α = 1.8%). Bacteria in stained smears showed an even

Table 3
Granulocyte elastase versus signs of inflammation

		Concentration range of granulocyte elastase (ng/ml)					
		0–249			250–999	1000–20000	
		n	n	%	n	n	%
signs of inflammation	no	38	29	32.6	5	4	12.2
	1	62	36	40.4	22	4	35.1
	2	33	17	19.1	8	8	21.6
	> 2	30	7	7.9	8	15	31.1
		163	89	100.0	74		100.0

more distinct relation ($\alpha = 0.13\%$) as outlined in detail in Table 1. Nevertheless, we flund bacteria in stained smears only in 17.6% of ejaculates with elevated elastase concentrations, that means in specimens of patients with conclusively ensured inflammation. On the other hand, bacteria in stained smears were also seen in ejaculates of patients without any inflammatory process.

Round cells and leukocytes: Round cell counts in ejaculates (n = 163) strongly correlated with increasing amounts of complexed elastase ($\alpha = 0.15\%$, Table 2). The same held true for leukocyte numbers ($\alpha = 0.02\%$). If the leukocytes are classified in "not pathologic" (0–2 cells per microscopic high power field) and "pathologic" (3 and more cells) and the elastase concentrations in "norm" (below 250 ng/ml) and "out of norm" (more than 250 ng/ml), the following allocation arises (Table 2): 17 (19.1%) of 89 ejaculates with normal elastase concentrations show more than 2 leukocytes per microscopic high power field, whereas 46 (62.2%) of 74 ejaculates with moderately or highly elevated amounts of complexed elastase contain leukocyte numbers in the normal range.

Quantification of the leukocyte counts with the Cyturtest demonstrated also a significant dependence on granulocyte elastase ($\alpha = 0.05\%$). However, due to only a few negative findings the results could be not assured statistically.

Inflammation coefficient: Statistical evaluation of "granulocyte elastase versus the inflammation coefficient" proved an especially marked correlation ($\alpha = 0.0002\%$) as outlined in Table 3. Whereas only in 12.2% of the 74 patients with elastase concentrations of 250 ng/ml and more no signs of inflammation according to our established coefficient could be found, 52.9% showed two and more signs of an inflammatory process. In contrast, in only 32.6% of the 89 patients with complexed elastase below 250 ng/ml no symptoms of an infection were seen, however, still 27% of this group demonstrated two and more signs of inflammation.

Discussion

Due to missing clinical symptoms silent male genital tract inflammation can be diagnosed only by laboratory parameters. Thus the presence of pathogenic microorganisms is taken as a proof for an inflammatory process. If microorganisms are not found, unspecific parameters such as the occurrence of increased numbers of leukocytes, pH-changes, liquefaction disorders, epithelial cells etc. are used so far as indicators of an ungoing inflammatory process. However, even in combination these factors gave no proof for silent male genital tract inflammation. Moreover, as a single parameter they are inadmissible for a

correct diagnosis. That seems to be true even for bacteria as shown in this study. With regard to granulocyte elastase as a highly sensitive and specific indicator of inflammation (Fritz et al. - 1984; Jochum et al. - 1985), in native preparations and stained smears of ejaculates bacteria were found only in less than one third of patients with elevated amounts of complexed elastase (more than 250 ng/ml) in their seminal plasma. This discrepancy might be explained by non-bacterial infections giving rise to an enhanced elastase release from stimulated polymorphonuclear granulocytes. On the other hand, the presence of bacteria in native ejaculates with low elastase concentrations may be due to contaminations during production of sperm.

Round cells in the native preparation are represented by leukocytes and immature cell stages of spermatozoa. Since most of the leukocytes are granulocytes and elastase is liberated during phagocytosis or disintegration of granulocytes, the strong correlation to the elastase amount in seminal plasma is not surprising. The same explanation should hold true for the even more pronounced correlation to the leukocytes in stained smears. Since the leukocyte count serves the attending doctor as an essential criterion of an inflammatory process, we quantified these cells in the ejaculates also with the Cyturtest. Thereby unspecific esterases derived from leukocytes are measured and a high correlation to the amount of granulocyte elastase in seminal plasma can be expected. However, the application of the test for analysis of seminal plasma is limited in its validity because of the following sources of error: In the ejaculate of even healthy persons up to 10000 non-pathogenic organisms/ml are found which probably give rise to false positive results. Furthermore, the important threshold for diagnosis of silent seminal tract infection is about 10^6 leukocytes/ml ejaculate whereas the Cyturtest gives a highly positive response even at 250 000 cells/ml urine. This high sensitivity may influence crucially the validity of the Cyturtest as a valuable method indicating inflammatory processes in the male efferent duct system. Since in this study the Cyturtest showed only a few negative findings, too, the results could be statistically not assured.

The especially high correlation of the quantity of elastase released into seminal plasma with the inflammation coefficient established by us can be taken as a further proof of granulocyte elastase being a highly specific marker of inflammatory reactions in the organism. If an inflammatory process is indicated by amounts of complexed elastase higher than 250 ng/ml and 2 signs of inflammation, respectively, still 35% of the patients classified "healthy" by the inflammation coefficient showed elastase concentrations indicative for an infection in the male efferent duct system. On the other hand, among the 63 patients classified with the usual sperm parameters to have "silent male genital tract inflammation", for 24 patients (38%) "no inflammation" was indicated because the elastase amounts were below 250 ng/ml. In our opinion estimation of granulocyte elastase for diagnosis of this clinically asymptomatic disease is clearly superior to the seminal parameters used up to now: It represents an essential simplification, since even a single determination gives a reliable criterion and due to an exact quantification it may allow the control of the course of the disease during therapy.

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