Cytokine Activation in Pericardial Fluids in Different Forms of Pericarditis

Sabine Pankuweit, Alexander Wädlich, Elmar Meyer, Irene Portig, Günter Hufnagel, Bernhard Maisch[1]

Abstract

There are many causes of pericardial effusion and it is useful to classify them etiologically, since this disorder is the most common pathologic process involving the pericardium. This report details our experience with pericardioscopy and epicardial biopsy in 101 patients with pericardial effusions in whom pericardioscopy was performed.

By means of clinical data and polymerase chain reaction we tried to elucidate the etiology of the pericardial effusion which were classified as follows: we found 41 effusions to be induced by primary malignant tumors or tumors metastatic to the pericardium. Specific diagnosis of viral and bacterial pericarditis was established in 17 patients by examination of the pericardial effusion with PCR, where we found 3 patients positive for adenovirus, 5 patients positive for cytomegalovirus, 2 patients positive for enterovirus-RNA and 5 patients positive for borrelia Burgdorferi-DNA. Additionally, idiopathic effusions (lymphocytic and autoreactive) were seen in 35 patients.

In summary immunological and molecular biology investigations seem to provide an additional tool in the diagnostic of pericardial effusion with unknown etiology. If we focus on the ELISA results, there is some evidence, that the demonstration of activation markers and soluble mediators of inflammation such as Il-6, Il-8 and IFN-gamma in pericardial effusion and the simultaneously lack of these mediators in sera of the patients first may be helpful in the discrimination of autoreactive and lymphocytic effusion. Second, this cytokine pattern or distribution indicates a possible local inflammatory process, where these cytokines were all released from activated T lymphocytes present in lymphocytic effusion. In the future, this may have therapeutic implications.

Pericardial effusion type		Infective (n = 17)	Malignant (n = 41)	Lymphocytic (n = 16)	Autoreactive (n = 19)
INF-gamma	PE	36.6	36.7	35.6	52.2
pg/ml	SE	66	51.6	19.4	51.4
IL-6	PE	2296.2	1937	1154.3	804.3
pg/ml	SE	1271.6	81.4	17.6	202.8
IL-8	PE	1111.4	1003.8	1203.9	689.8
pg/ml	SE	734	42.9	2.3	205

Key Words: Pericardial effusion · Cytokines · Il-6 · Il-8 · TNF-alpha · PCR

Zytokinaktivierung im Perikarderguss bei Patienten mit unterschiedlichen Formen einer Perikarditis im Vergleich zum Serum

Zusammenfassung

Die Ursachen für eine Perikarditis mit Ergussbildung sind unterschiedlich, sodass es sinnvoll ist, diese nach ihren Ätiologien zu unterscheiden.

In diese Untersuchung wurden 101 Patienten mit Perikarditis und Ergussbildung unterschiedlicher Ätiologie eingeschlossen, wobei jeweils eine Perikardioskopie mit Punktion des Ergusses und Perikard- bzw. Epikardbiopsie durchgeführt wurde. Neben den klinischen wurden labordiagnostische Untersuchungen zur Klärung der Ursache der Ergussbildung durchgeführt. Hierzu gehörte der Nachweis von Infektionserregern über Polymerasekettenreaktion (PCR)

Perikarderguss-typ		Infektiös (n = 17)	Maligne (n = 41)	Lymphozytär (n = 16)	Autoreaktiv (n = 19)
INF-gamma	PE	36,6	36,7	35,6	52,2
pg/ml	SE	66	51,6	19,4	51,4
IL-6	PE	2296,2	1937	1154,3	804,3
pg/ml	SE	1271,6	81,4	17,6	202,8
IL-8	PE	1111,4	1003,8	1203,9	689,8
pg/ml	SE	734	42,9	2,3	205

[1]Department of Internal Medicine – Cardiology, Philipps-University Marburg, Germany.

aus dem Erguss der Patienten ebenso wie zytologische Untersuchungen.

Die Patienten konnten so in folgende Gruppen eingeteilt werden: 41 Ergüsse wurden durch maligne Tumoren oder Tumormetastasen hervorgerufen. Die spezifische Diagnose einer viralen oder bakteriellen Perikarditis konnte durch PCR aus der Perikardflüssigkeit bei 17 Patienten nachgewiesen werden. Idiopathische Ergüsse (lymphozytär/autoreaktiv) wurden bei 35 Patienten gefunden. Um mögliche Hinweise auf autoreaktive Mechanismen zu erhalten, wurden im Vergleich zum Serum Zytokine (Interferon gamma, Interleukin 6 und 8) mittels ELISA nachgewiesen.

Die Ergebnisse weisen darauf hin, dass der Nachweis von Aktivierungsmarkern und löslichen Mediatoren der Entzündungsreaktion wie Il-6, Il-8 und IFN-gamma im Perikarderguss und das gleichzeitige Fehlen dieser Mediatoren im Serum der Patienten zunächst einmal zur Unterscheidung zwischen lymphozytären und autoreaktiven Perikardergüssen hilfreich sein kann. Zweitens deutet diese Zytokinverteilung auf einen lokalen entzündlichen Prozess mit Freisetzung der Zytokine aus aktivierten T-Zellen hin, der möglicherweise in Zukunft therapeutische Konsequenzen haben könnte.

Schlüsselwörter: Perikarderguss · Zytokine · Il-6 · Il-8 · TNF-alpha · PCR

There are many causes of pericardial effusion and it is useful to classify them etiologically, since this disorder is the most common pathologic process involving the pericardium [3, 32]. They include first viral, bacterial, fungal or rickettsial infections, second non-infectious diseases like acute myocardial infarctions, radiation, trauma, storage diseases, neoplastic disorders and third pericardial effusion presumbly related to hypersensitivity and autoimmunity for example in rheumatoid arthritis [8, 29].

This report details our experience with pericardioscopy and epicardial biopsy in 101 patients with pericardial effusions in whom pericardioscopy was performed.

By means of clinical data, polymerase chain reaction (PCR) and immunohistochemistry we tried to elucidate the etiology of the pericardial effusion [15, 17, 21, 24], which were classified as follows: we found 41 effusions to be induced by primary malignant tumors or tumors metastatic to the pericardium (Table 1). Specific diagnosis of viral and bacterial pericarditis was established in 17 patients by examination of the pericardial effusion with PCR, where we found 3 patients positive for adenovirus, 5 patients positive for cytomegalovirus, 2 patients positive for enterovirus RNA and 5 patients positive for borrelia Burgdorferi DNA. Further on, we found 3 patients with pericardial effusion after cardiac injury and 5 patients with non-infectious effusions caused by uremia. Additionally, idiopathic effusions were seen in 35 patients. The large proportion of idiopathic effusions demonstrates the lack of diagnostic tools capable of defining the etiology and pathogenesis of these diseases.

For this we investigated pericardial effusion and sera of the patients by the use of enzyme-linked immunosorbent assay for interferon gamma (IFN-gamma), interleukin 6 (IL-6) and interleukin 8 (IL-8) to detect feasible markers for the discrimination of idiopathic autoreactive and lymphocytic pericardial effusion [14]. All of these cytokines have been described in association of certain autoimmune and infectious diseases [30], they are all released from activated T and B cells and monocytes, present the idiopathic pericardial effusion. IL-6 is one of the important cytokines for the stimulation and differentiation of B cells resulting in secreting of specific antibodies. IL-8 as a member of the chemokine family acts as a chemotractant and activating factor for neutrophils and enhances the adherence of neutrophils to

Etiology	No. of patients	Method
Malignant tumor or tumor metastasis	41	Clinical data + cytology
Viral pericarditis	10	PCR
– Adenovirus	3	
– Cytomegalovirus	5	
– Enterovirus	2	
Bacterial pericarditis	7	PCR + serology
– Borrelia Burgdorferi	5	
– Others	2	
Uremia	5	Clinical data
Postcardiac injury	3	Clinical immunological data
Idiopathic	35	Immunological analysis

Table 1
Etiology of the pericardial effusion.

Tabelle 1
Ätiologie der untersuchten Perikardergüsse.

endothelial cells by inducing the expression of adhesion molecules. INF-gamma is the most important activating factor for macrophages with antiviral and antiproliferative activity and enhances the expression of major histocompatibility Class 1 and Class 2 molecules.

Demonstration and quantitation of these cytokines in pericardial effusion and sera of the patients may contribute to a better understanding of the immunopathologic mechanismens possibly involved in the pathogenesis of chronic idiopathic pericardial effusion.

Patients and Methods

This report included 101 patients with pericardial effusion undergoing pericardioscopy and epicardial or pericardial biopsy. Immediately after puncture and biopsy the samples (e. g. pericardial effusion, epi/pericardial biopsy, serum) were snap-frozen and stored at -80 °C till the day of investigation. Small samples of pericardial effusion and epi/pericardial biopsy were investigated for the presence of malignant cells or lymphocytes by cytological techniques.

By means of clinical data, PCR and immunohistochemistry we tried to elucidate the etiology of the pericardial effusion [16, 17]. For this we looked at immunological parameters which may be helpful in the diagnosis of possible autoreactive, antibody-mediated or lymphocytic pericardial effusions. This includes determination of antisarcolemmal and antimyolemmal antibodies, immunohistochemistry of the peri/epicardial biopsy for the detection of infiltrating cells and the PCR for the detection of cardiotropic viruses in the biopsies.

Immunofluorescence and Immunohistochemistry Analyses

Antisarcolemmal (ASA) antibodies (covalently fixed at the biopsy) and antimyolemmal (AMLA) antibodies (present in sera or pericardial effusion) were detected by direct and indirect immunofluorescence technique with the epicardial biopsy and isolated cardiomyocytes as antigens [19, 20].

Immunohistochemistry was performed with the epicardial or pericardial biopsy after cutting them in 5 µm thin sections. For demonstration of infiltrating cells we used antibodies specific for activated T and B cells (CD2, CD3, CD4, CD8, CD45), macrophages (CD11c, CD14), major histocompatibility Class-1 and Class-2 antigens (HLA-ABC, HLA-DR, HLA-DP, HLA-DQ), adhesion molecules (ICAM-1) and endothelial cells (EN 4). Specific binding of the antibodies was demonstrated by peroxidase staining procedure [10, 12, 20].

Molecular Biological Analyses

For demonstration of cardiotropic viruses to be present in the epicardial/pericardial biopsy or the pericardial effusion total DNA and RNA was extracted from the samples [5, 13]. Primer pairs specific for the following gene segment were used for amplification and/or reverse transcription [2, 4, 9, 11, 23, 26, 27]:

	Size of the PCR-product
1. region "5'NTR" for enterovirus	198 bp,
2. region "US10/11" for cytomegalovirus	360 bp,
3. region "Hexon" for adenoviruses	308 bp,
4. region matrix protein for influenzaviruses	625 bp,
5. region DNA polymerase for herpes simplex viruses	229 bp.

Ten microliters of each reaction were analyzed on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. All samples were run with a simultaneous positive and negative control for the virus analyzed [25]. Southern blotting and hybridization with specific probes was used to confirm positive results. For each primer pair a digoxigenin labeled hybridization probe was available.

Enzyme Linked Immunosorbent Assay (ELISA)

For the quantitation of cytokines (e. g. IL-6, IL-8, IFN-gamma) sera and pericardial effusion were stored immediately after recovery at -80 °C in aliquots to prevent degradation of the proteins [28, 30]. We used quantitative 2-step colorimetric sandwich ELISAs (Quantikine™, R & D systems) that contains recombinant standards. The kits recognized natural IFN-gamma, IL-6 and IL-8 in different samples with a sensitivity of less than 3 pg/ml (IFN-gamma), less than 0.9 pg/ml (IL-6) and less than 10 pg/ml (IL-8). Sera taken from healthy controls were used as negative control samples. Optical density of each well was determined using a microplate reader set to 450 nm. Wavelength correction was set to 570 nm.

The average of duplicate readings for each standard, control and sample were subtracted from the average of the zero standard optical density. After generation of the standard curve concentration of each set of samples could be read easily.

Results

Specific diagnosis of viral and bacterial pericarditis was established in 17 patients by examination of the pericardial effusion with PCR (Table 1), where we found 3 patients positive for adenovirus, 5 patients positive for cytomegalovirus, 2 patient positive for enterovirus RNA and 5 patients positive for borrelia Burgdorferi DNA. An example of the PCR for the detection of enteroviral RNA and cytomegaloviral DNA is shown in Figures 1a and 1b.

By the use of cytological and immunohistochemical analyses we found the following results: In lymphocytic effusions by definition lymphocytes were the dominant cell type, but polymorphs were also observed. In viral and autoreactive antibody-mediated effusions the number of lymphocytes was substantially reduced. No changes in lymphocyte number of peripheral blood were observed. Neoplastic cells were only detected in malignant effusions.

Antisarcolemmal antibodies were seen in all forms of effusion, but antimyolemmal antibodies with higher titers were only detected in viral and autoreactive effusions. Figures 2a and 2b show an example of antisarcolemmal antibodies detected with immunofluorescence technique by use of an epicardial biopsy as antigen. Antibodies in the pericardial effusion from a patient with autoreactive effusion were directed against structures of the sarcolemm. Antibody-mediated cardiocytotoxicity of the pericardial effusion in the presence of a fresh complement source was primarily detected in autoreactive effusion.

Infiltrates (Figure 2b) in the epicardial biopsies consisting of lymphocytic and mononuclear cells were seen

Figures 1a and 1b
Results of the molecular biological investigation of the pericardial/biopsy/pericardial fluid. a) Detection of cytomegalovirus DNA; b) detection of enterovirus RNA.

Abbildungen 1a und 1b
Ergebnisse der molekularbiologischen Untersuchungen an Epikardbiopsien bzw. Perikarderguss. a) Nachweis von Zytomegalievirus-DNA; b) Nachweis enteroviraler RNA.

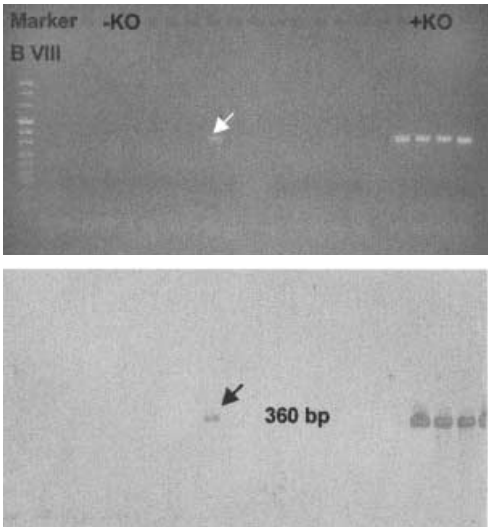

Figure 1a

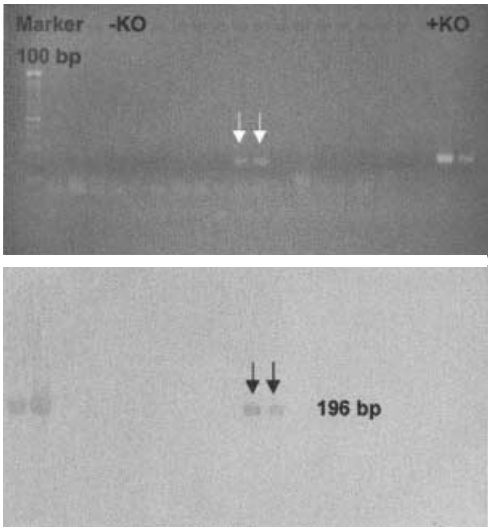

Figure 1b

Figures 2a and 2b
Immunofluorescence (a) and immunohistochemical (b) investigations by use of an epicardial biopsy as antigen. a) Detection of antisarcolemmal antibodies (magnification 400x). b) Detection of infiltrating CD3 and CD4 positive cells (magnification 630x).

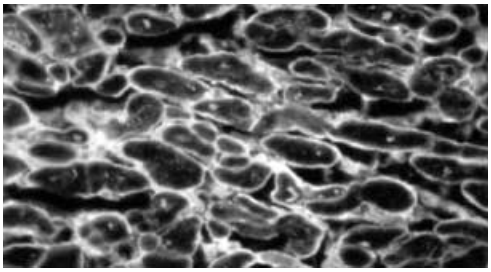

Figure 2a

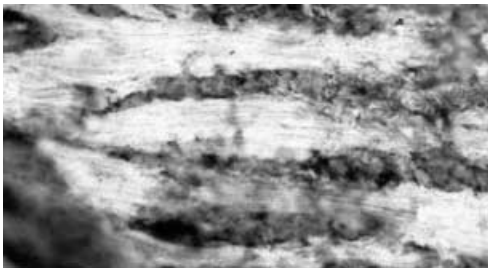

Figure 2b

Abbildung 2a und 2b
Immunfluoreszenz- (a) und immunhistochemische (b) Untersuchungen an Epikardbiopsien. a) Nachweis antisarkolemmaler Antikörper (400-fache Vergrößerung). b) Nachweis infiltrierender CD3- und CD4-positiver Zellen (630fache Vergrößerung).

in 90% of patients with lymphocytic effusion and in 50% of patients with viral effusions but only few in the possibly humoral induced autoreactive form. In this biopsy infiltrating cells were CD3 and CD4 positive.

If we focus on the ELISA results (Figure 3) we found strongly elevated levels of IFN-gamma in sera of patients with the viral induced form (65 pg/ml) in contrast to the serum of these patients (35 pg/ml) and only slightly elevated levels of IFN-gamma in effusions and sera of patients with lymphocytic (38 pg/ml effusion, 19 pg/ml serum), autoreactive (52 pg/ml effusion, 51 pg/ml serum) and malignant (38 pg/ml effusion, 51 pg/ml serum) induced pericardial effusion. The expression of this molecule in sera of control persons ranges between 15 to 20 pg/ml. This was the first remarkable observation.

Il-6 was strongly elevated in pericardial effusions of all patients (ranging from 650 to 2,300 pg/ml) and the sera of patients with viral (1,270 pg/ml) and autoreactive (262 pg/ml) induced forms except the sera of patients with malignant (51 pg/ml) and lymphocytic (17 pg/ml) effusions. The same was true for Il-8, which was strongly elevated in sera and effusions of all patients (ranging from 265 to 1265 pg/ml) except the sera of patients with malignant (42 pg/ml) and lymphocytic (2.5 pg/ml) effusions. None of these cytokines could be detected in sera of control persons.

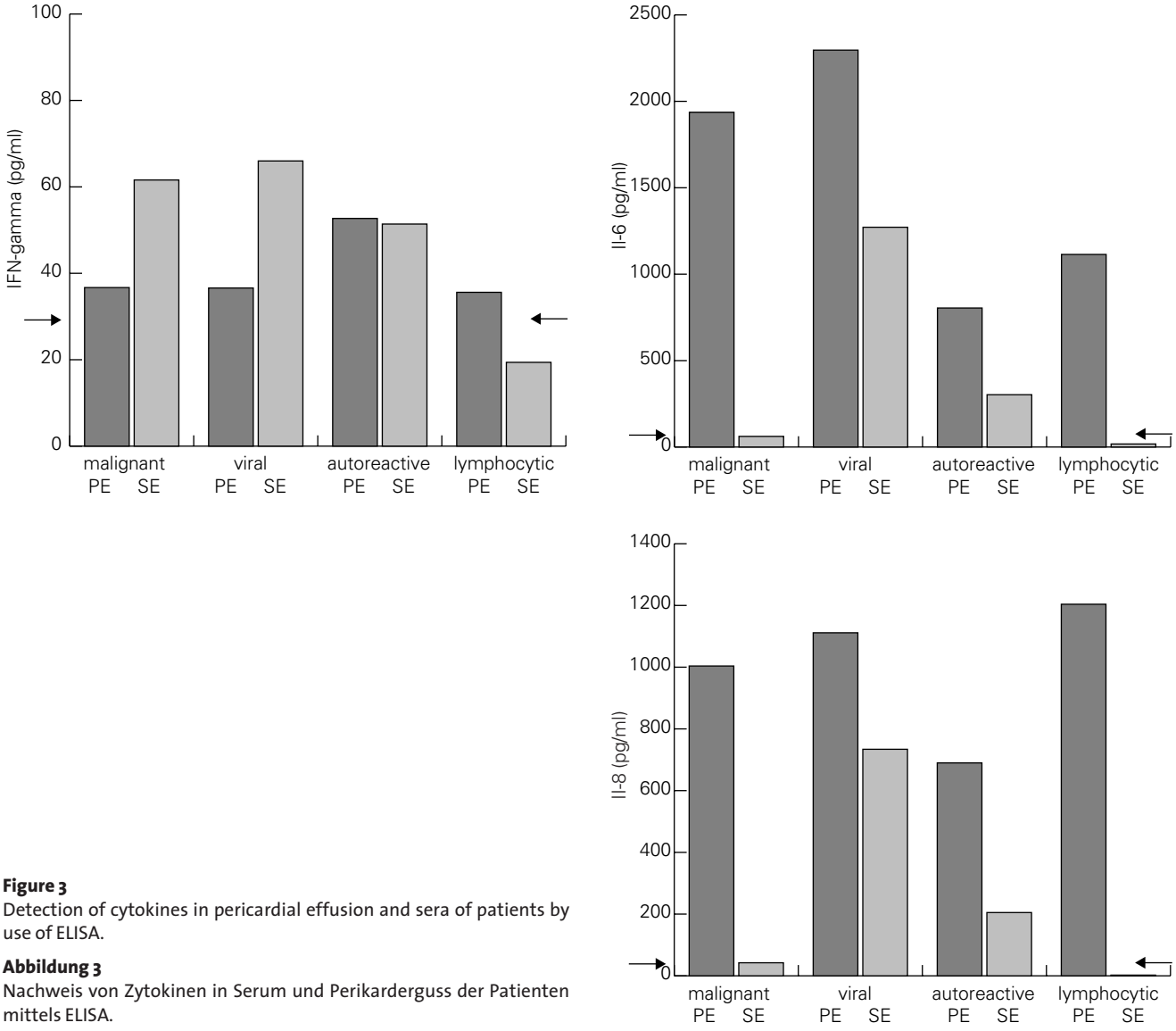

Figure 3
Detection of cytokines in pericardial effusion and sera of patients by use of ELISA.

Abbildung 3
Nachweis von Zytokinen in Serum und Perikarderguss der Patienten mittels ELISA.

Conclusions

In summary immunological and molecular biology investigations seem to provide an additional tool in the diagnostics of pericardial effusion with unknown etiology. The diagnosis of viral and bacterial effusions has become easier by the use of techniques like PCR [18], were we can use a lot of different primer pairs for the diagnosis of viral and bacterial infections. Usage of this method together with the assessment of pericardial effusion and/or pericardial and epicardial effusion [18, 33] helps us to discriminate patients with an infectious etiology of the disease clearly from those with malignant disorders and those with idiopathic effusion.

The entity of so-called idiopathic effusion except the infectious or malignant induced forms should be divided in the possible autoreactive, humoral induced forms and the lymphocytic induced forms. We can detect lymphocytic effusions by demonstration of infiltrating lymphocytes in the pericardial effusion and the epicardial biopsy on condition that the biopsy is taken from the lesion of interest. Immunoserology is of even greater sensitivity for an inflammatory or autoreactive antibody-mediated process, because these antimembrane antibodies were detected in high levels in autoreactive induced effusions. Immunosuppressive therapy in patients with autoreactive effusion for example with triamcinolone in a crystalloid suspension given intrapericardially as previous reported or installation of an anti-OKT3 antibody in case of the proven lymphocytic effusion in patients with perimyocarditis may provide new specific therapeutic options [1, 7, 18, 31]. If we focus on the ELISA results, there is some evidence that the demonstration of activation markers and soluble mediators of inflammation such as Il-6, Il-8 and IFN-gamma in pericardial effusion and the simultaneously lack of these mediators in sera of the patients first may be helpful in the discrimination of autoreactive and lymphocytic effusion. Second, this cytokine pattern or distribution indicates a possible local inflammatory process, where these cytokines were all released from activated T lymphocytes present in lymphocytic effusion [30]. Further investigations including the establishment of a human pericardial fluid bank [6] may help us in understanding possible immunological mechanisms being involved in the pathogenesis of pericardial effusion.

Acknowledgements:

The authors acknowledge the kind support given by the Department of Pathology (Head: Prof. Dr. Moll) and the Department of Hematology (Prof. Dr. Neubauer, PD Dr. Wolf) Philipps University Marburg, who have done the cytological analyses of possible malignant pericardial effusion.

References

1. Berman J, Haffajee CI, Alpert JS. Therapy of symptomatic pericarditis after myocardial infarction: retrospective and prospective studies of aspirin, indomethacin, prednisone, and spontaneous resolution. Am Heart J 1981;101:750–3
2. Bowles NE, Richardson PJ, Olsen E, et al. Detection of Coxsackie B virus specific RNA sequences in myocardial biopsy samples from patients with myocarditis and dilated cardiomyopathy. Lancet 1986;1:1120–3.
3. Butany J. The pericardium and its diseases. In Silver DM, ed. Cardiovascular pathology. New York, NY: Churchill Livingstone, 1991: 847–94.
4. Chapman NM, Tracy S, Gauntt CJ, et al. Molecular detection and identification of enteroviruses using enzymatic amplification and nucleic acid hybridisation. J Clin Microbiol 1990;28:843–50.
5. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–9.
6. Dichinson TJ, Gurudutt V, Ngyen AQ, et al. Establishment of a clinically correlated human pericardial fluid bank: evaluation of intrapericardial diagnostic potential. Clin Cardiol 1999;22:Suppl 1:I-40–2.
7. Garg A, Shiau J, Guyatt G. The ineffectiveness of immunosuppressive therapy in lymphocytic myocarditis: an overview. Ann Intern Med 1998;129:317–22.
8. Goodie RB. Secondary tumors of the heart and pericardium. Br Heart J 1955;17:183–92.
9. Goodman JL, Jurkovic P, Kramber JM, et al. Molecular detection of persistent borrelia burgdorferi in the urine of patients with active Lyme disease. Infect Immunol 1991;59:269–78.
10. Hengstenberg C, Rose ML, Olsen EGJ, et al. Immune response to the endothelium in myocarditis, dilated cardiomyopathy and rejection after heart transplantation. Eur Heart J 1991;12:Suppl D:144–6.
11. Hufnagel G, Busse C, Ische B, et al. Detection of adenoviral DNA in endomyocardial biopsies of adult patients with myocarditis and dilated cardiomyopathy. Eur Heart J 1997;18:Suppl 515.
12. Hufnagel G, Maisch B. Expression of MHC class I and II antigens and the IL-2 receptor in rejection, myocarditis and dilated cardiomyopathy. Eur Heart J 1991;12:Suppl D:137–40.
13. Jackson DP, Lewis FA, Taylor GR, et al. Tissue extraction of DNA and RNA and analysis by polymerase chain reaction. J Clin Pathol 1990;43:499–504.
14. Kelly RA, Smith TW. Cytokines and cardiac contractile function. Circulation 1997;95:778–81.
15. Maisch B, Bethge C, Drude L, et al. Pericardioscopy and epicardial biopsy – new diagnostic tools in pericardial and perimyocardial disease. Eur Heart J 1994;15:Suppl C:68-73.
16. Maisch B, Drude L. Epi- and pericardial biopsy by pericardioscopy. Circulation 1992;82:Suppl 3:417.abstract 1658.
17. Maisch B, Drude L. Pericardioscopy – a new diagnostic tool in inflammatory diseases of the pericardium. Eur Heart J 1991;12:Suppl D:2–6.
18. Maisch B, Pankuweit S, Brilla C, et al. Intrapericardial treatment of inflammatory and neoplastic pericarditis guided by pericardios-

copy and epicardial biopsy – results from a pilot study. Clin Cardiol 1999;22:Suppl 1:I17–22.
19. Maisch B, Schönian U, Bittinger A. Increased diagnostic sensitivity of epimyocardial biopsy in comparison to endomyocardial biopsy in perimyocarditis. Eur Heart J 1994;15:Suppl A:948.
20. Maisch B, Schönian U, Herzum M, et al. Immunoserologic and immunohistologic studies of myocarditis and pericarditis. Internist (Berlin) 1995;36:448–57.
21. Maisch B. Pericardial diseases, with a focus on etiology, pathogenesis, pathophysiology, new diagnostic imaging methods, and treatment. Curr Opin Cardiol 1994;9:379–88.
22. Millaire A, Wurtz A, de Groote P, et al. Malignant pericardial effusions: usefulness of pericardioscopy. Am Heart J 1992;124:1030–4.
23. Mockenhaupt T, Reschke M, Bogner E, et al. Structural analysis of the US-segment of a viable temperature sensitive human cytomegalovirus mutant. Arch Virol 1994;137:161–9.
24. Nugue O, Millaire A, Porte H, et al. Pericardioscopy in the etiologic diagnosis of pericardial effusion in 141 consecutive patients. Circulation 1996;94:1635–41.
25. Pankuweit S, Hufnagel G, Eckhardt H, et al. Cardiotropic DNA viruses and bacteria in the pathogenesis of cardiomyopathy with and without inflammation. Med Klin 1998;93:223–33.
26. Piiparinen H, Vaheri A. Genotyping of herpes simplex viruses by polymerase chain reaction. Arch Virol 1991;119:541–8.
27. Schönian U, Crombach M, Maisch B. Assessment of cytomegalovirus DNA and protein expression in patients with myocarditis. Clin Immunol Immunopathol 1993;68:229–33.
28. Schönian U, Paul R, Gemsa D, et al. Soluble IL2-receptor-, IL6 and TNF a in inflammatory pericardial effusions. Eur Heart J 1995;16: Suppl A:P1711.
29. Scott RW, Garvin CF. Tumors of the heart and pericardium. Am Heart J 1939;17:431–45.
30. Schreiner GF. Immune modulation of cardiac cell function. Trans Am Clin Climatol Assoc 1998;109:39–49.
31. Seferovic PM, Ristic AD, Maksimovic R, et al. Initial clinical experience with PerDUCER®: Promising new tool in the diagnosis and treatment of pericardial disease. Clin Cardiol 1999;21:30–5.
32. Spodick DH. The pericardium: a comprehensive textbook. 1st ed. New York: Marcel Dekker, 1997:260.
33. Westaby S. Pericardioscopy – a new approach in the management of pericardial effusions of unknown etiology. Proc Br Cardiol Soc 1990;17:114–5.

Address for Correspondence:
Dr. rer. physiol. Sabine Pankuweit, Department of Internal Medicine – Cardiology, University, Baldinger Straße, D-35043 Marburg, Germany,
Phone (+49/6421) 286-6462, Fax -8954,
e-mail: pankuwei@mailer.uni-marburg.de