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| Administration: | Dr M. Keipes, secrétaire général<br>Dr P. Burg, assistant au secrétaire<br>Clinique Ste-Thérèse,<br>36, rue Zithe, L-2763 Luxembourg<br>Tél: ++352 497 766 363<br>Fax: ++352 497 764 949<br>GSM: ++352 091 199 733<br>E-mail: mkeipes@hotmail.com<br>Compte en banque:<br>Dexia LU14 0024 1014 1150 0000<br>CCPL LU 1111 0004 4860 0000 |
| Rédaction: | Dr G. Berchem, CHL,<br>4, rue Barblé, L-1210 Luxembourg<br>E-mail: berchem.guy@chl.lu |





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# Sommaire

# 25 ans de sport pour Cardiaques à Luxembourg
# Développement d'un modèle de rééducation durable

*Charles Delagardelle et Patrick Feiereisen*

*Service de Cardiologie du Centre Hospitalier de Luxembourg*

**Abstract:**

*25 years of organized ambulatory heart sport in Luxembourg*

*The development of a sustained rehabilitation model*

After Second World War cardiovascular disease (CVD), especially coronary artery disease (CAD), turned out to be an epidemic in the western countries including the Grand-duchy of Luxembourg, and accounted for nearly half of all deaths. A lot of strategies, among them treatment by physical activity, were developed to fight this challenge and, fortunately, a mortality regression of about 50 % could be achieved. Nowadays, eastern European countries and, more recently, China and India face an increasing CVD mortality.

During the seventies ambulatory heart sport clubs, then labeled as, "coronary clubs" became very popular in Europe especially in West-Germany. Around 2000 there were more than 6000 heart sports groups in Germany. In 1984 a first group was founded in Luxembourg City (Centre) a, 1991 a second group started in Esch/Alzette (South) and in 2002 a third regional group in Ettelbruck (North) so that, by now, the 3 main public health districts of the Grand-Duchy of Luxembourg can offer regular ambulatory sports activities to almost all concerned cardiac patients in the country. The ambulatory heart sport groups of Luxembourg cooperate in a federated association allowing an integrated logistic organization.

Since the beginning nineties cardiac rehabilitation became a field of interest to the university faculties and later of scientific societies, like the American Heart Association (AHA) and the European Society of Cardiology (ESC). New subgroups were founded and guidelines were published and renewed. The movement of ambulatory heart sport groups was more or less neglected in the prevention and rehabilitation activities of the scientific societies.

Recently the ESC proposed a new definition of comprehensive CVD prevention and rehabilitation programmes as "coordinated, multifaceted interventions designed to optimize a cardiac patient's physical, psychological, and social functioning, in addition to stabilizing, slowing, or even reversing the progression of the underlying atherosclerotic processes, thereby reducing morbidity and mortality". The responsible ESC cardiologists agree with the international community that

fighting CVD risk factors is at least as important as the whole arsenal of modern heart surgery and interventional cardiology.

The core activity of ambulatory heart sport groups remains physical activity, and nowadays 6 different activities can be offered (one activity each day of the week): exercise lesson, swimming, walking, cycling, Nordic Walking and water gymnastics On the other hand comprehensive prevention programs, especially concerning CVD risk factors are also endorsed by the ambulatory heart sport groups of Luxembourg via regular meetings, conferences, brochures and symposia. One advantage of the ambulatory heart sport movement in Luxembourg, in contrast to the German model, is the direct financial allowance of the health ministry, which permits a lifelong activity to all the active members. Another advantage is that all the regional groups are directed by clinical cardiologists knowing the patients very closely. One weak point is that only about 5-10% of all potential candidates adhere to the ambulatory heart sport groups but nearly 50 % of the active members are practicing for more than 5 years. These regularly active patients are a positive selection of well committed cardiac patients who, most of the time, control CVD risk factors with scrutiny.

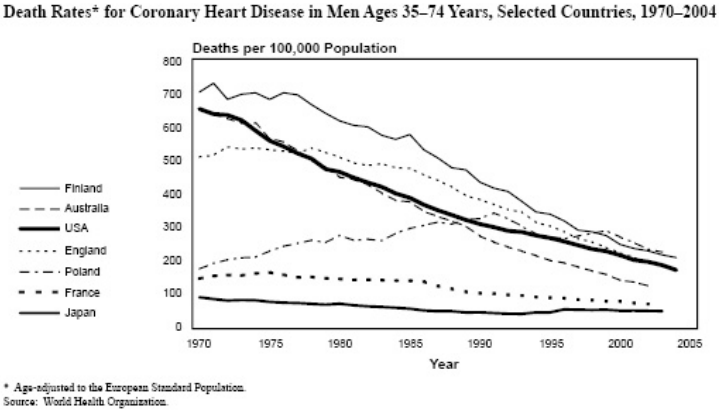
The ESC has recommended creating so called "Heart Houses" where all the aspects of comprehensive prevention and rehabilitation can be offered. Their main concern is to develop a sustained strategy which is desperately missing for the moment. A lot of the active members of the heart sport groups of Luxembourg achieve such a sustained activity and, therefore, these heart sport groups can be considered as very cost effective models of sustained rehabilitation.

After a 25 years activity the ambulatory heart sport movement of Luxemburg has reached the outstanding goal of being a center of sustained rehabilitation. Although such a goal was not really planned during the first 15 years of activity, the stamina of the active members set the movement into this direction.

**Key Words:** ambulatory heart sport groups, 25 years activity, regional groups, comprehensive and sustained rehabilitation,

Après la deuxième guerre mondiale la maladie coronarienne s'est répandue comme une vraie épidémie tout d'abord aux Etats-Unis, plus tard en Europe de l'ouest. Des efforts énormes ont été entrepris pour combattre cette maladie, qui était rapidement devenue la cause numéro 1 de morbidité et de mortalité. Dans un premier temps on a identifié les principaux facteurs de risque (FR) que sont l'hypercholestérolémie, l'hypertension artérielle (HTA), le tabagisme, le diabète type 2 et la sédentarité. Tous ces FR sont plus ou moins directement liés au style de vie plus en plus sédentaire qui s'était progressivement installé, favorisé par le progrès technique et l'avènement des automobiles. Grâce aux multiples efforts entrepris par la communauté scientifique, en association avec les responsables politiques, on a réussi à endiguer cette épidémie et, surtout, à diminuer la mortalité chez les patients de moyen âge (Graphiques 1,2 )(1).

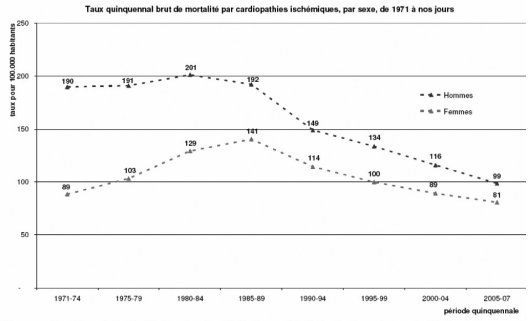
**Graphique 1 : Mortalité par maladie coronarienne (hommes) dans différents pays**




*Ce graphique montre l'évolution de la mortalité coronarienne dans différents pays avec une incidence de maladie coronarienne très variable : La Finlande, l'Autriche et les Etats-Unis avec une mortalité très élevée en 1970 suivie d'une diminution spectaculaire*

Depuis 1980 on assiste à une remonté de la même pathologie dans les pays de l'Europe de l'est et depuis l'an 2000 dans les grands pays de l'Asie notamment l'Inde et la Chine, qui regroupent plus d'un tiers de la population mondiale. D'aucuns prévoient pour ces 2 pays une épidémie encore plus grave que celle d'après guerre dans nos pays et dont les causes sont par ailleurs identiques (2).

**Graphique 2 : Évolution de la mortalité coronarienne au Luxembourg (Hommes et femmes)**




**Source** : Direction de la Santé, Service des Statistiques, Statistiques des causes de décès

*Au Grand-duché la mortalité était également très élevée en 1970. Par rapport aux années 1980 on a observé une diminution de la mortalité de > 50% chez les hommes et de > 43 % chez les femmes.*

Depuis une cinquantaine d'années l'entraînement physique, en prévention primaire, et la rééducation cardiaque en prévention secondaire, se sont progressi-

vement développés et ont été reconnus comme interventions très efficaces pour combattre les maladies cardio- vasculaires dégénératives (1, 3)

Déjà en 1968 l'Organisation Mondiale de la Santé (OMS) avait proposé une organisation de la réadaptation cardiaque et cette classification en 3 phses reste toujours actuelle en 2010 : Phase 1 ou phase aigue qui va jusqu'à 2-3 semaines après un syndrome coronarien aigu..Phase 2 ou phase de convalescence qui va jusqu'à 10 semaine et finalement phase 3 ou phase d'entretien qui, théoriquement, se prolonge toute la vie. C'est dans la phase 3 que se situent les activités des groupes sportifs pour cardiaques, qui, à partir de 1970 ont été créés en Allemagne.

Au Grand-duché de Luxembourg le sport pour cardiaques a été organisé de manière systématique à partir de 1984 c.-à-d. depuis 25 ans Dans le temps il s'agissait d'une des premières mesures systématiques de médecine préventive dans notre pays (4).

## 1. Le développement scientifique de la prévention compréhensive

En parallèle avec l'extraordinaire développement de la cardiologie clinique, la recherche fondamentale a également fait des progrès énormes Elle a entraîné une vraie éclosion de littérature scientifique dans toutes les sous-spécialités cardiologiques y inclus la prévention primaire et secondaire et la rééducation des pathologies cardiaques par activité physique.

Pendant la première phase du sport pour cardiaques ambulatoire de 1965-1985, celui-ci était considéré comme moderne, quasi révolutionnaire, et a engendré un grand nombre de publications scientifiques. Cependant à partir de 1985 on a observé une dissociation entre la recherche fondamentale et le mouvement des groupes sportifs pour cardiaques, qui pourtant, surtout dans les pays germanophones –rien qu'en Allemagne il existe plus de 6000 groupes - n'est pas négligeable. La cause principale de cette évolution a été la constatation que la seule intervention par entraînement sportif est insuffisante pour influencer le pronostic des patients, et qu'il faut une intervention également au niveau des autres FR. Depuis les années 80 une attention particulière a été attribuée au mode de vie (« lifestyle ») incluant les problèmes psychologiques, professionnels et nutritionnels (5). Certes le FR « sédentarité » y garde toute son importance mais il n'est qu'un facteur parmi d'autres. Le concept de *la rééducation cardiaque compréhensive* a été développé. Il prévoit une intervention au niveau de *tous* les FR et fait le joint entre activités sportives, interventions comportementales et médicamenteuses. Depuis une dizaine d'années le diabète type 2 et l'obésité ont été associés comme importantes cibles d'intervention. Une autre explication de cette dissociation est le fait que les structures organisationnelles des groupes sportifs pour cardiaques ne sont plus directement liées aux facultés universitaires et celles-ci ont ensuite progressivement délaissé le mouvement du sport pour cardiaques.

Aujourd'hui le domaine de la rééducation et de la prévention secondaire a été pris en main par la communauté scientifique très active des sociétés cardiologiques savantes américaines (American Heart Association AHA) (6) et européennes (European Society of Cardiology, ESC) (7), qui ont élaboré des *« guidelines »* (« lignes de conduite »). Elles concernent le domaine de la rééducation cardiaque et de la prévention secondaire reconnus comme deux éléments essentiels et indissociables.

La *réhabilitation (rééducation)* y est définie de la façon suivante : *Intervention multiple, coordonnée pour optimiser le fonctionnement physique, psychologique et social des patients. En plus : stabiliser, retarder et même renverser la progression du processus athéromateux pathologique sous jacent et, ainsi, réduire la morbidité et la mortalité.( 7)*

Les programmes des guidelines concernant la prévention secondaire comportent un examen clinique, des recommandations diététiques, un traitement agressif des FR (lipides, tabac, hypertension, poids et diabète), des conseils psychosociaux et d'intégration professionnelle, ainsi que des conseils sur l'activité physique et l'entraînement. La prescription de médicaments cardio-protecteurs, dont l'efficacité dans la prévention secondaire a été prouvée par la médecine factuelle (« evidence based medecine »). En d'autres mots, l'amélioration des FR joue un rôle essentiel dans les guidelines.

Il s'agit d'une définition complexe qui, en fait, englobe la quasi totalité de ce que l'on peut faire pour améliorer le pronostic des patients cardiaques. Les conseils pour une activité physique régulière et les recommandations d'entraînement ne sont que des éléments parmi beaucoup d'autres. En pratique l'activité physique et l'entraînement sont considérés comme des surplus qui s'ajoutent au traitement médicamenteux et au régime, contribuant ainsi à augmenter non seulement le nombre des années, mais surtout de la qualité de vie des années ajoutées (2, 6, 7. 8)

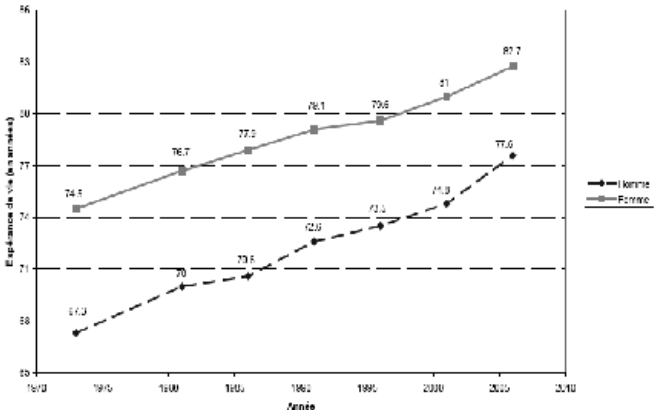
Au sein des groupes sportifs pour cardiaques le sport joue, historiquement parlant, le rôle primordial  mais les autres facteurs de la prévention ne sont pas négligés pour autant. Même si la première mission du mouvement sportif pour cardiaques reste toujours la pratique de sports bien adaptés de façon durable, si possible jusqu'à la fin de la vie, tous les autres éléments de la prévention secondaire sont également  pris en considération.

## 2. Le problème de la durabilité de la prévention cardiaque

En analysant les progrès formidables de la cardiologie récente on aurait tendance à croire que l'amélioration du pronostic est en premier lieu imputable aux nouvelles thérapies invasives c.-à-d. les opérations de pontage et les dilatations coronariennes. Cependant la recherche épidémiologique récente, moyennant des techniques statistiques très poussées, a pu montrer que ces progrès thérapeutiques ne peuvent être attribués même pas pour la moitié au progrès du traitement invasif, mais à plus de 50% à l'intervention au niveau des FR (9,10). En d'autres mots *les*

*FR sont effectivement une cible thérapeutique très importante et prioritaire.* En revenant aux guidelines et aux analyses critiques sur les effets concrets de la « rééducation compréhensive » on peut conclure que, malgré des résultats partiellement acceptables au niveau du traitement médicamenteux de l'hypercholestérolémie, il reste beaucoup de travail à faire. Dans le contexte de l'espérance de vie qui augmente, on va avoir beaucoup plus de cardiaques âgés car cette amélioration de la survie entraîne un déplacement des problèmes vers un âge plus élevé (Graphique 3). Dans le même ordre d'idées on comprend pourquoi le nombre d'hospitalisations pour problèmes cardiaques et de patients en insuffisance cardiaque sont en train d'augmenter considérablement. Cette évolution démographique entraîne une énorme hausse du coût pour les assurances de maladie et les systèmes sociaux.

**Graphique 3 Evolution de l'espérance de vie à Luxembourg** **STATEC6/2008**




*Sur le graphique 3 on voit 'augmentation de l'attente de vie continuelle que nous sommes en train de vivre et est en toute première ligne dû aux progrès dans la cardiologie Au Luxembourg l'âge moyen est actuellement de 81 ans pour les femmes et de 78 pour les hommes (1, 9,10)*

Un des moyens le plus efficaces et le moins onéreux pour combattre le problème est la rééducation par le sport. Les responsables de la section prévention et réhabilitation de la Société Européenne de Cardiologie en sont pleinement conscients et ils font un plaidoyer en faveur de la création de *centres de prévention primaire et secondaire* permettant d'appliquer les guidelines de façon plus effective et surtout pendant une période beaucoup plus longue (11). Même s'ils accordent une petite mention des groupes sportifs pour cardiaques dans leurs analyses, on a l'impression qu'ils sous-estiment les possibilités de ces derniers.

Dans les guidelines proprement dit, auxquelles on reproche fréquemment qu'elles sont trop extensives, les groupes sportifs pour cardiaques ne sont plus mentionnés du tout. Ceci est regrettable car le mouvement du sport ambulatoire pour cardiaques dispose d'une expérience pratique de plus de 40 ans et pourrait offrir un élément qui fait cruellement défaut dans la réhabilitation des cardiaques c.-

à-d. que *l'intervention soit durable à long terme.* Dans les guidelines on reprend surtout des études, certes bien planifiées selon les règles de la médecine factuelle, mais concernant presque toutes la période précoce après un évènement cardiaque aigu et avec un temps d'intervention souvent très (trop) court. Aussi bien aux Etats-Unis qu'en Europe les sociétés scientifiques déplorent, en premier lieu, qu'un très grand pourcentage de patients ne fait pas de rééducation du tout - 80 % aux E-U et en moyenne 70 % dans les divers pays européens. Ensuite elles déplorent que cette rééducation, qui devrait être une intervention définitive, à vie, n'est pas poursuivie de façon durable (6, 7, 8).

Des analyses critiques concernant l'efficacité de la prévention et de la réhabilitation dite « compréhensive » telle quelle est préconisée dans les guidelines, comme les études EUROASPIRE I-III (8) montrent que beaucoup de patients candidats ne participent pas du tout à une rééducation ou bien pendant une période trop courte et, comme conséquence, la plupart des patients ne réussissent pas d'atteindre les buts fixés.

Pour le Grand-duché de Luxembourg la situation générale concernant la rééducation cardiaque est assez favorable et on peut estimer que *70-80% de tous nos patients cardiaques susceptibles subissent une rééducation.* En effet depuis une bonne dizaine d'années la rééducation ambulatoire intra-hospitalière phase 2, est en progression constante Plusieurs cliniques grand-ducales ont mis sur pied une rééducation ambulatoire pour les patients récemment hospitalisés et, actuellement, on peut offrir un tel traitement dans les 3 districts sanitaires (sud, nord et centre) du Grand-duché :

A part les patients plus jeunes, qui, souvent, préfèrent la rééducation ambulatoire il y a un assez grand nombre de patient(e)s plus âgé(e)s et nécessitant une infrastructure hospitalière complète, est transféré dans des cliniques spécialisées à l'étranger pour y subir une rééducation dite stationnaire.

## 3. Analyse critique du sport pour cardiaques à Luxembourg

Depuis le début de l'ALGSC les responsables n'ont pas hésité à se mettre en question. (4,5) *Tous les 5 ans, lors des journées du sportif cardiaque,* des analyses critiques ont été présentées.

En résumant la *situation de l'ALGSC en 2010* on peut dire après une activité de 25 ans, que le but primaire, qui était d'offrir aux patients cardiaques luxembourgeois un forum pour un entraînement physique adapté et contrôlé, a été atteint. Dans les 3 districts du secteur médical luxembourgeois il y a une section régionale de sport pour cardiaques car, entretemps en 2001, la section nord d'Ettelbruck a été fondée et elle a connu un développement spectaculaire. Un élément clef du succès d'Ettelbruck est l'engagement de tout un groupe de cardiologues qui adressent leurs patients de façon systématique et leur engagement pourra servir de modèle

aux autres sections. En effet dans la région centre il y a une clinique très active et deux autres qui le sont nettement moins et dans la région sud, qui, concernant le potentiel en patients est certainement la plus importante, il n'y a qu'un seul cardiologue très actif

Ceci explique en grande partie pourquoi le but d'inclure un grand pourcentage de patients cardiaques luxembourgeois, susceptibles de participer aux activités sportives de phase 3, n'a pas été atteint. D'un autre côté il faut souligner dans ce contexte que *l'entraînement physique constitue l'élément le plus dur de la rééducation cardiaque*. En effet il est beaucoup plus simple de prendre tous les jours plusieurs des médicaments pour diminuer le taux de cholestérol ou la tension artérielle, que de se surmonter et pratiquer du sport.

L'avènement des *centres de rééducation ambulatoire phase 2* dans plusieurs cliniques luxembourgeoises, qui, en principe, constitue une évolution très favorable, est devenu une arme à double tranchant pour les groupes sportifs pour cardiaques. Alors que ces centres de phase 2 pourraient être une excellente méthode de recrutement pour les groupes sportifs de phase 3 on n'en a pas su tirer le bénéfice escompté. Une des explications pour cette tendance est que les programmes de phase 2 sont individualisés et la prise en charge des patients y est personnalisée. Alors que l'entraînement en groupe était moderne jusqu'il y a 20 ans, le sport pour cardiaques subit aujourd'hui les mêmes changements qu'on peut observer au niveau de tout le mouvement du sport loisir c.-à-d. une nette tendance vers l'entraînement personnel, si possible sur machines dans une salle de fitness. Une partie non négligeable de patients cardiaques optent pour cette filière, mais, malheureusement, faute de motivation, *la majorité des patients va arrêter* tout simplement les activités sportives.

En analysant les chiffres du Centre hospitalier de Luxembourg (CHL) où chaque année environ 200 patients suivent une rééducation ambulatoire phase 2, on compte en moyenne seulement 12 nouvelles recrues par an pour la section Luxembourg, c.-à-d. *seulement 6 % de candidats* potentiels vont suivre la rééducation à long terme, durable phase 3 En d'autres mots, il y a un vrai déséquilibre entre la phase 2 de la rééducation cardiaque, qui s'étend jusqu'à la 12$^{e}$ semaine après un évènement cardiaque aigu et la phase 3, la phase chronique, qui théoriquement va jusqu'à la fin de la vie.

## 4. Modèle Luxembourgeois : Les groupes sportifs centres de prévention

Le mouvement du sport pour cardiaques luxembourgeois, qui, au départ, a imité le modèle allemand a développé assez rapidement son propre style. Il y a plusieurs raisons à cela :1) Contrairement au modèle allemand les activités pour nos sportifs pour cardiaques ne sont pas limitées dans le temps. *Notre modèle de financement direct par le ministère de la santé* nous permet d'offrir à nos sportifs une activité à vie. 2) Les 3 sections régionales de l'ALGSC sont affiliées à un centre cardiologique

régional et elles sont dirigées par des cardiologues expérimentés qui connaissent les sportifs. Soulignons encore une fois que l'engagement des cardiologues est cependant très variable; excellent dans la section nord, moyen dans la section centre et insuffisant dans la section sud. Ces cardiologues sont directement impliqués dans l'organisation des groupes qui, en Allemagne sont organisés par différents clubs sportifs et financés par les caisses de maladie. 3) a partir de 1990 on a élargi nos activités sportives en ajoutant d'abord la natation, ensuite le cyclisme et la marche, à partir de 2001 le « nordic walking » (NW) et à partir de 2005 l'aquagym. En d'autres mots : on peut offrir 6 disciplines sportives pendant 6 jours de la semaine 4) Les 3 sections disposent de très bonnes structures organisationnelles. Celles-ci sont assurées par les patients eux-mêmes qui constituent le comité central et les comités régionaux. *Ces gens bénévoles sont en fait les piliers du succès depuis 25 ans.*

Dans la *section centre il y a actuellement, en 2010, 90 sportifs actifs* régulièrement. : 65 font de la gym dont 15 font également du NW, 14 du cyclisme, 14 de la natation et 5 de l'aquagym. 8 sportifs du centre ne pratiquent que la natation et 8 également que du NW, la plupart pour des raisons orthopédiques (Graphique 4)

Plus que la moitié des sportifs pratiquent au moins, et ceci de façon régulière, deux fois par semaine, une douzaine 3, voire 4 disciplines par semaine.

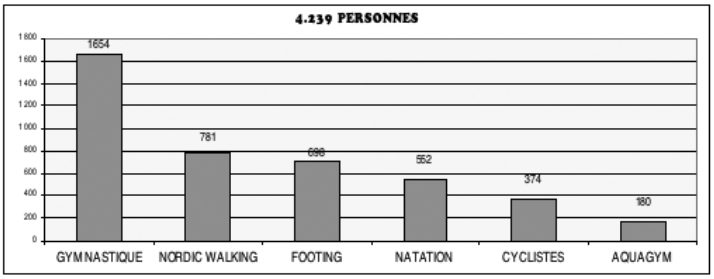
Parmi ces 90 sportifs «réguliers » 12 pratiquent depuis plus de 10 ans, 24 depuis plus de 5 ans et 18 depuis plus de 3 ans

Au fil des années s'est développé un groupe de sportifs très actifs, remplissant sans doute les critères des sociétés savantes au niveau de l'activité physique, mais également très consciencieux les autres FR :

*En considérant la durée des activités on constate que 70 % remplissent également le critère probablement le difficile à réussir, de la durabilité*

Lors des analyses précédentes notre ambition principale a été surtout le nombre de nouveaux sportifs. Au vu du nombre de patients qui subissent une rééducation phase 2, ce nombre devrait se situer, rien que pour le CHL (200 patients/an en phase 2), entre 50-80 nouveaux recrus pour les groupes sportifs pour cardiaques par an. En considérant qu'il n'y a que 10-12 nouveaux sportifs/an on est donc très loin de ce chiffre

**Graphique 4 : Activités sportives de la section Luxembourg en 2009**



*Les activités sportives ont changé fondamentalement depuis une quinzaine d'années. S'il est vrai que les séances hebdomadaires de gymnastique restent l'activité la plus fréquente, 40 % de toutes les séances, les autres activités sportives font 60 % des séances totales : le NW 18%, le footing 16 %, la natation 14 %, le cyclisme 8 % et l'aquagym 4 %.*

Un problème majeur est la constatation que certains groupes de patients sont sous représentés ; seulement 15% de nos actifs sont des femmes et il y a seulement 2 sportifs d'origine portugaise parmi globalement 35 % de résidents non Luxembourgeois

Pendant les 25 ans écoulés on a organisé, à un rythme régulier, un grand nombre de conférences, surtout sur les FR et la nutrition, mais aussi sur les différentes pathologies cardio-vasculaires et les nouveautés thérapeutiques. A part cela on a offert des cours pratiques de diététique et des cours de premier secours pour les patients et leurs partenaires de vie. La fréquentation de ces organisations était globalement excellente, permettant de conclure qu'il y a un vrai besoin dans le domaine. Très souvent les conjointes (conjoints), et les anciens sportifs cardiaques, qui pour la plupart, restent des membres dits « non-actifs », se sont associés à ces manifestations. Même si on n'a pas un niveau de clinique ou de centre spécialisé, on a su offrir à nos sportifs une éducation valable. Notre expérience de 25 ans permet de conclure qu'il y a un vrai besoin dans le domaine

## 5. En résumé

Pendant les 15 premières années de notre activité on poursuivait surtout le but de faire rentrer un maximum de patients au sein de l'ALGSC. Au fil du temps nous avons changé, sans nous en rendre compte et sans l'avoir planifié au départ, en un cercle de sportifs cardiaques très appliqués et disciplinés à long terme. Il y a eu un vrai *changement de paradigme* qu'on pourrait résumer simplement en disant qu'on *travaille dans la qualité plutôt que dans la quantité.* Dans ce contexte il est important de souligner qu'on n'est vraiment pas un cercle fermé et que nos portes restent grandes ouvertes à de nouveaux membres.

Au Grand-duché les adhérents aux groupes ne sont certes pas assez nombreux, mais ils/elles sont fidèles et assidu(e)s. Il s'agit d'une sélection positive de patients très conformes aux prescriptions (« compliant »), qui, hélas, constitue une minorité parmi les nombreux patients cardiaques.

Tout compte fait on peut être fier après 25 ans d'activités. *Le modèle Luxembourgeois du sport pour cardiaques* a atteint un grand nombre des critères stipulés par les autorités pour les centres de prévention. Pour les années à venir on essayera de continuer dans la même voie et d'améliorer encore les structures organisationnelles, entre autre pour organiser des séances d'information concernant les FR de façon encore plus systématique. Une condition, sine qua non, pour réussir sera de trouver assez de bénévoles pour assurer le travail au sein des comités. Nous espérons également de trouver de jeunes cardiologues, kinésithérapeutes, infirmières pour compléter nos équipes multidisciplinaire

# Littérature

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# Predicting significant fibrosis in hepatitis C patients in Luxembourg using serological markers

*Joël Mossong[1],*, Stéphanie Bill[2], Karin Hawotte[3], Georges Gilson[2], Ulrich Knolle[1], Joseph Weber[2], Tania Roskams[4], Vic Arendt[2]*

*[1] Laboratoire National de Santé, Luxembourg*
*[2] Centre Hospitalier de Luxembourg, Luxembourg*
*[3] Centre de Recherche Public – Santé, Luxembourg*
*[4] Department of Morphology and Molecular Pathology, Katholieke Universiteit Leuven, Belgium*

** Corresponding author address & address for reprints: Joël Mossong, Surveillance & Epidemiology of Infectious Diseases, Laboratoire National de Santé, PO Box 1102, L-1011 Luxembourg. Tél : 00352 491191-336. Fax: 00352 404238. Email:joel.mossong@lns.etat.lu*

## Short title: Predicting fibrosis in hepatitis C patients

**Abstract**

Objective: The aim of our study was to assess the diagnostic performance of various serological markers and scores for predicting significant fibrosis retrospectively in a population of patients referring to our hospital for liver biopsy and chronic hepatitis C. Materials and Methods. Stored serum obtained from 186 patients were tested for a number of biological markers putatively associated with liver fibrosis. Fibrotest and Forns scores were compared with liver fibrosis pathology scored according to the METAVIR system by multiple logistic regression. Results: The prevalence of significant fibrosis was 44%. Aspartate amino transferase (AST) and γ-glutamyltransferase (GGT) were most correlated with METAVIR staging, followed by platelet counts and $\alpha_2$-macroglobulin. The negative predictive value was 77% and 83% and the positive predictive value was 100% and 84% for the Forns score and the Fibrotest, respectively. In multivariate analysis AST, GGT and $\alpha_2$-macroglobulin had independent predictive power. Conclusions: The accuracy of serological markers in predicting significant fibrosis is limited, because approximately two thirds of patients lie into an indeterminate "grey zone". Serological markers might be useful for patients reluctant to undergo liver biopsy but current predictive scoring systems are too inaccurate to replace biopsies in a routine manner.

**Keywords :** *Hepatitis C, fibrosis, serological markers, METAVIR, Forns, Fibrotest*

## Introduction

Hepatitis C is a major public health problem. The World Health Organization estimates the prevalence of hepatitis C infection to be approximately 1% of the population [1]. About 75-85% of infected patients will become chronic carriers

and have an increased risk of developing liver cirrhosis and liver cancer. Current treatment has many side effects and is expensive. Hence the decision for initiating treatment for individual patients is based on staging of hepatic fibrosis as determined by liver biopsies. But the liver biopsy is an invasive procedure and can be associated with pain, complications and mortality as well substantial societal costs.

In recent years, several biological, biochemical, serological and biophysical surrogate markers have been proposed to help predict the stage of fibrosis [2-8]. In a review, Gebo et al. suggested that biochemical blood tests and serologic tests have a modest value in predicting fibrosis on liver biopsy but that a combination of markers have the greatest predictive value [9].

The aim of our study was to assess the diagnostic performance for predicting significant fibrosis of two predictive scores (the Fibrotest [5] and the score proposed by Forns [4]) retrospectively in a population of patients referring to our hospital for liver biopsy and chronic hepatitis C. Furthermore we assessed the diagnostic performance of some additional biochemical markers not included in the panel of markers of the two above scores.

## Methods

### Patients

The study population consisted of 186 patients followed for chronic hepatitis C at the departments of infectious diseases or gastroenterology at the Centre Hospitalier de Luxembourg from 1994 to 2004. All patients with chronic hepatitis C confirmed by two positive anti-HCV serologies and with an available liver biopsy specimen and stored serum contemporary to the biopsy were included in the study.

### Serological markers and laboratory testing

The following markers were tested concurrently to the biopsy (aspartate amino transferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transferase (GGT), platelet count, prothrombine time, alkaline phosphatase (PAL), cholesterol, bilirubin, albumin). The following markers were tested in 2004 (alpha-2-macroglobulin, hyaluronic acid (Corgenix, Westminster, USA), TIMP-1 (BioSource International Inc., Camarillo, USA), cholinesterase, apolipoprotein a1, haptoglobin) on stored serum samples. Viral loads were performed using the COBAS Amplicor (Roche Diagnostics, USA) and genotyping by the Tru-Gene HVC 5'NC methodology (Bayer Healthcare, Germany)..

### Histology

Liver biopsy specimens were initially analysed after biopsy by a group of general pathologists at the National Health Laboratory in Luxembourg. Specimens were reanalyzed retrospectively by one of the authors (TR) considered an expert liver

pathologist. In case of discordant scoring, the scoring of the expert pathologist was used for further analysis. Fibrosis was staged according to the METAVIR scoring system [10].

### Predictive diagnostic scores

The Forns score, f, was calculated using the published formula

$f = 7.811 - 3.131 * \ln(\text{platelets}) + 0.781 * \ln(\text{GGT}) + 3.467 * \ln(\text{age}) - 0.014 * \text{cholesterol}$

Scores <4.2 were considered to be indicative of no significant fibrosis (METAVIR 0 or 1), scores in the range 4.2-6.9 were considered to be equivocal and scores >6.9 were considered to be indicative of advanced fibrosis (METAVIR 2, 3 or 4).

Results for the Fibrotest were obtained from Biopredictive for 133 samples. Scores less than or equal to 0.1 were considered to be indicative of no significant fibrosis (METAVIR 0 or 1), scores >= 0.6, scores in the range 0.1-0.6 were considered equivocal. On a subsample of 53 patients the Fibrotest score was estimated by multiple logistic regression using the parameter estimates published in the patent [11].

### Statistics

Multiple logistic regression was performed to assess the diagnostic performance of biological and serological markers. Exact singly ordered Kruskal-Wallis test was used to test an association between METAVIR scores and genotypes (categorized into genotype 1 - genotypes 2, 3, 4 and unknown genotype) using StatXact-4 (Cytel Software Corporation).

## Results

### Patient characteristics

The study population consisted of 67 (36%) female and 119 (64%) male patients with confirmed chronic hepatitis C infection. Mean age was 39 years (range 17-76, inter-quartile range 30-45). Genotyping was available for 132 patients and genotypes 1, 2, 3 and 4 were were found in 79 patients (60%), 4 patients (3%), 37 patients (28%) and 7 patients(5%), respectively. The distribution of the METAVIR score by the expert pathologist was as follows: no fibrosis in 13 patients (F0=7%); portal fibrosis in 92 patients (F1=49%), few septa in 43 patients (F2=23%), numerous septa in 28 patients (F3=15%) and cirrhosis in 10 patients (F4=5%). The prevalence of significant fibrosis (F2, F3, F4) was therefore 44%.

### Comparison of histology

Comparative histological scoring was available for 168 patients. 70 (42%) biopsies had concordant Metavir scores, 82 (49)% had a metavir discordance of 1 unit and 16 (9%) had a Metavir discordance of 2 or 3 units. Overall 36 (21%) biopsies

had a METAVIR discordance which would lead to different therapeutic outcome (i.e. score of 0 or 1 by one pathologist and score of 2 or higher by the other pathologist. The METAVIR scoring by the expert pathologist had a higher correlation with the Fibrotest (Cuzick's test for trend, $z=6.13$, $p<0.001$ for expert pathologists and $z=4.87$, $p<0.001$ for routine pathologists ) and with the Forns score (Cuzick's test for trend, $z=6.55$, $p<0.001$ for expert pathologists and $z=5.87$, $p<0.001$ for routine pathologists). Based on these results, the expert pathologist's METAVIR score was used for all further analyses.

### Univariate correlation with markers

Table 1 shows the univariate correlation of serological and biological markers against the METAVIR score as assessed by Cuzick's test for trend. The liver enzymes AST and GGT had a highly significant correlation, followed by platelet counts and $\alpha$-2 macroglobulin. Further significant markers for METAVIR stage were age, TIMP-1, ALT and hyaluronic acid and to a lesser extent haptoglobulin, cholinesterase, prothrombin time, apolipoprotein A1, total cholesterol and viral load. No significant correlation was found for PAL, sex, bilirubin and albumin. Figure 1 shows boxplots of of AST and TIMP-1. Whereas the association of METAVIR score with AST is linear, the correlation with TIMP-1 only appears to be substantially higher for cirrhosis. There was no association of METAVIR score with genotype (Kruskal-Wallis test, $p=0.38$).

### Estimated Fibrotest

Based on results from 133 patients, the Fibrotest, f, was estimated to be as follows:

$$f = \exp(\delta) / \exp(\delta + 1) \text{ where}$$

$$\delta = 4.4888*\log(a2macro) - 1.3713*\log(hapto) + 1.0051*\log(GGT) + 1.6699*\log(bilirubin) - 1.1992*APO + 0.0279*age + 0.2933*sex - 5.3979$$

These estimated parameter values are very close to the parameter values published in the patent [11] and indeed lie within the indicated uncertainty range. Figure 2 shows the actual Fibrotest results against the estimated scores indicating an extremely high correlation ($R2=0.998$). Based on this high correlation, we estimated the Fibrotest scores for a further 53 patients using the above formula.

### Diagnostic performance of Forns & Fibrotest

The diagnostic performance of the Fibrotest and Forns scoring systems are shown in table 2 and 3, respectively. The ROC curve analyses gave an area under the curve of 0.74 and 0.73 and these were not significantly different (figure 3). The negative predictive value at the lower cutoff was 77% for the Forns and 83% for the Fibrotest. 35 (19%) patients had a Fibrotest score of <0.1 and for them liver biopsy could have been avoided. Of these, 6 (17%) had a false-negative result with a METAVIR score of F2 being observed. 43 (23%) had a Forns score <4.2 and

of these 10 (23%) had a false negative result, 7 patients with a F2 and 3 patients with F3.

The positive predictive value at the higher cut-off was 100% and 84% respectively. 31 patients had a Fibrotest score >0.6 (17%) of whom 5 (16%) had a false positive results, all with a METAVIR score of F1. The Forns test identified 17 (9%) patients with significant fibrosis with 100% accuracy.

However, 65% and 67% of patients had scores in the equivocal range for the Fibrotest and Forns, respectively.

**Multivariate analysis and estimation of a new score.**

The following variables identified in the univariate analysis as significant predictors of fibrosis were tested in a multivariate logistic model: AST, GGT, platelets, A-2 macroglobulin, age, TIMP-1, ALT, hyaluronic acid, haptoglobulin and total cholesterol. As shown in table 1, only 3 variables had independent predictive power: AST, GGT and A-2 macroglobulin. It is interesting to note that the high predictive value of AST was conditional on removing 5 samples from the analysis with extremely low value below 10. The area under the ROC-curve of this model was 0.84 (96% CI 0.78-0.90.

The formula for this score is:

$$s = exp(\delta) / exp(\delta + 1), \text{ where}$$

$$\delta = 3.8298*log(AST) + 3.6224*log(a2macro) + 1.6931*log(GGT) - 11.4096$$

As for the Fibrotest and Forns score, two cut-off values can be chosen to indicate absence and presence of significant fibrosis. With the lower cut-off of 0.3, we would identify 86% of 77 patients correctly as not having significant fibrosis (negative predictive value). With the upper cut-off of 0.6, we would identify 85% of 55 patients with significant fibrosis (positive predictive value). Using such a scheme would enable a prediction of fibrosis with approximately 85% accuracy in 132 (73%) of all patients.

## Discussion

Our study had a number of weaknesses. First the study design was retrospective with patients recruited over an eleven year period and part of the serological testing was done several years after sample collection based on stored serum samples. It is unclear to what extent the storage might have affected the results of the biochemical tests. For this reason, the TGF-beta marker could not be used in our study. Also like almost all studies on predicting fibrosis in hepatitis C patients, patient data on duration of infection, alcohol consumption and previous treatment was too incomplete to be included in the analysis.

Second, compared to other studies, our sample size was moderate and insufficient to have a validation group for an independent assessment of a new diagnostic

score including AST. Furthermore, the Fibrotest was estimated for a subsample of patients, although correlation of estimated and real Fibrotest scores was very high ($R^2$=0.998).

We found a certain amount of inter-observer variability between histological METAVIR scoring, but the fact that we are comparing a group of general pathologists and one expert liver pathologist makes this finding difficult to interpret. The expert liver pathologist's scoring tended to be higher (mean 1.6) than the scoring of general pathologists (mean 1.0) in our group of patients and we used the expert pathologist's scoring for the remainder of our study. This finding suggests that it is important to involve expert liver pathologist in studies on liver fibrosis.

Our study confirms that some serological markers used in the Fibrotest or Forns score are associated with significant fibrosis: notably GGT, platelet counts and α-2 macroglobulin. We have found in addition that AST is independently associated with significant fibrosis as has been found by other investigators [12-14], whereas the correlation of fibrosis with ALT was less significant. TIMP-1 and hyaluronic acid also correlate in univariate analysis but do not have a significant independent correlation in the multivariate analysis. As such there is probably limited value of adding these two fibrosis specific markers to routine work up of patients with hepatitis C.

Our study suggests that the value of Fibrotest & Forns score in predicting absence or presence of significant fibrosis is limited. With either score, about two thirds of patients fall into the "grey zone" where no prediction is possible. Our findings show that diagnostic accuracy can be improved to a moderate extent by adding AST in the list of serological markers such that one might be able to avoid 73% of biopsies. However one would have to accept that about one in 6 patients, the prediction of fibrosis would be inaccurate such that either unnecessary treatment would be initiated or treatment withheld when it was indicated.

We conclude that certain serological markers might be useful for patients which are reluctant to undergo liver biopsy but that current predictive scoring systems based on serological markers are too inaccurate to replace biopsies in a routine manner. Whether other newly introduced approaches like measuring liver stiffness [15] could improve diagnostic accuracy remains to be seen.

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**Table 1:** Correlation of significant fibrosis with serological markers – univariate analysis.

| Marker | Sample size | Cuzick's \|z\| value | p-value (univariate) |
|---|---|---|---|
| AST | 186 | 5.73 | <0.001 |
| GGT | 186 | 5.65 | <0.001 |
| Platelets | 186 | 4.94 | <0.001 |
| $\alpha_2$-macroglobulin | 186 | 4.69 | <0.001 |
| Age | 186 | 4.14 | <0.001 |
| TIMP-1 | 183 | 4.03 | <0.001 |
| ALT | 186 | 3.58 | <0.001 |
| Hyaluronic Acid | 132 | 3.41 | <0.001 |
| Haptoglobulin | 186 | 3.00 | 0.003 |
| Cholinesterase | 135 | 2.84 | 0.005 |
| Prothrombin time | 135 | 2.72 | 0.006 |
| Apolipoprotein A1 | 185 | 2.55 | 0.011 |
| Total cholesterol | 186 | 2.20 | 0.028 |
| Viral load | 163 | 2.08 | 0.034 |
| PAL | 120 | 1.74 | 0.082 |
| Sex | 186 | 1.49 | 0.136 |
| Bilirubin | 186 | 0.90 | 0.37 |
| Albumin | 133 | 0.13 | 0.894 |

**Table 2:** Diagnostic performance of the Forns score in predicting significant fibrosis for different cut-offs.

| Score cutoff | Sensitivity | Specificity | Correctly classified | Likelihood ratio | PPV | NPV |
|---|---|---|---|---|---|---|
| 0.1 | 92.59% | 27.62% | 55.91% | 1.2792 | 50% | 83% |
| 0.2 | 77.78% | 60.00% | 67.74% | 1.9444 | | |
| 0.3 | 61.73% | 74.29% | 68.82% | 2.4005 | | |
| 0.4 | 46.91% | 82.86% | 67.20% | 2.7366 | | |
| 0.5 | 39.51% | 89.52% | 67.74% | 3.771 | | |
| 0.6 | 32.10% | 94.29% | 67.20% | 5.6173 | 84% | 65% |
| 0.7 | 22.22% | 96.19% | 63.98% | 5.8333 | | |
| 0.8 | 13.58% | 99.05% | 61.83% | 14.2593 | | |
| 0.9 | 7.41% | 100.00% | 59.68% | ∞ | | |

Area under the curve: 0.74 (95% CI 0.67-0.81)

**Table 3:** Diagnostic performance of the Forns scores in predicting significant fibrosis.

| Score cutoff | Sensitivity | Specificity | Correctly classified | Likelihood ratio | PPV | NPV |
|---|---|---|---|---|---|---|
| 1.00 | 94% | 9% | 46% | 1.0262 | | |
| 2.00 | 90% | 24% | 53% | 1.1829 | | |
| 3.00 | 80% | 52% | 65% | 1.6852 | | |
| 4.00 | 60% | 72% | 67% | 2.1903 | | |
| 4.20 | 58% | 77% | 69% | 2.5386 | 50% | 77% |
| 5.00 | 49% | 87% | 70% | 3.7037 | | |
| 6.00 | 32% | 96% | 68% | 8.4259 | | |
| 6.90 | 22% | 100% | 66% | ∞ | 100% | 62% |

Area under the curve: 0.73 (95% CI 0.66-0.81)

**Table 4 :** Results when applying multiple logistic regression on complete data set (N=179 with non-missing data) for significant fibrosis.

| Variable* | β (SE) | p-value |
|---|---|---|
| Age | .086 (1.523) | 0.955 |
| AST | 3.364 (.945) | <0.0001 |
| GGT | 1.615 (.607) | 0.008 |
| Platelets | -1.685 (1.546) | 0.276 |
| A-2 macroglobulin | 3.692 (1.176) | 0.002 |
| Haptoglobulin | -1.130 (.914) | 0.216 |
| TIMP-1 | 1.176 (.889) | 0.186 |
| Constant | -9.882 (5.513) | 0.073 |

* All variables were log-transformed prior to analysis.

Hosmer-Lemeshow goodness-of-fit test on 10 groups: p=0.55.

**Figure 1.** Box plots of a) AST and b) TIMP-1 as a function of METAVIR score.

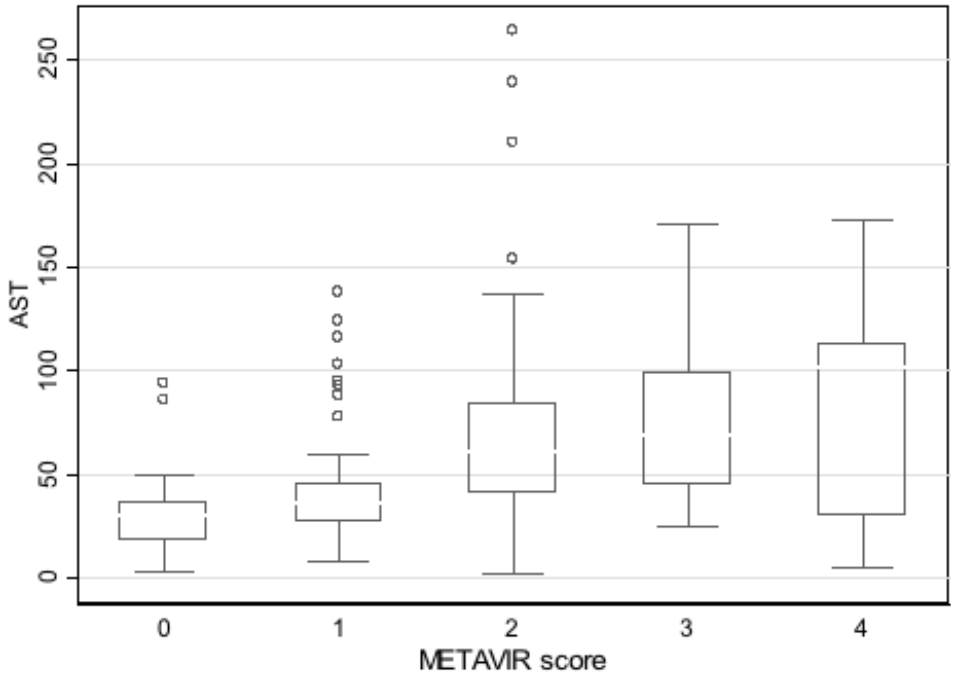



**Figure 2.** Correlation of actual and estimated Fibrotest.

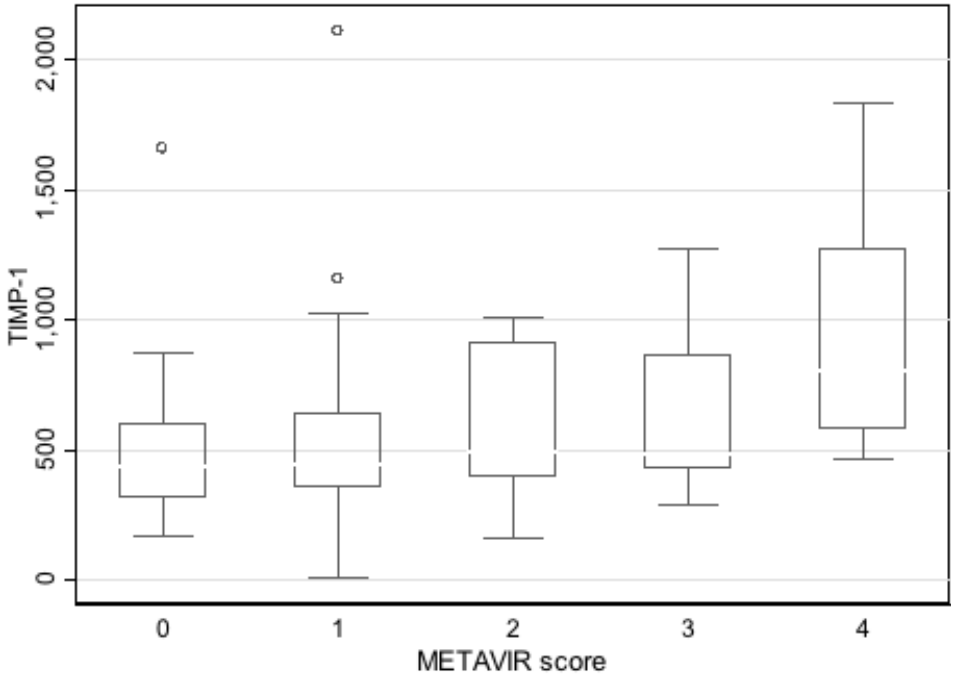
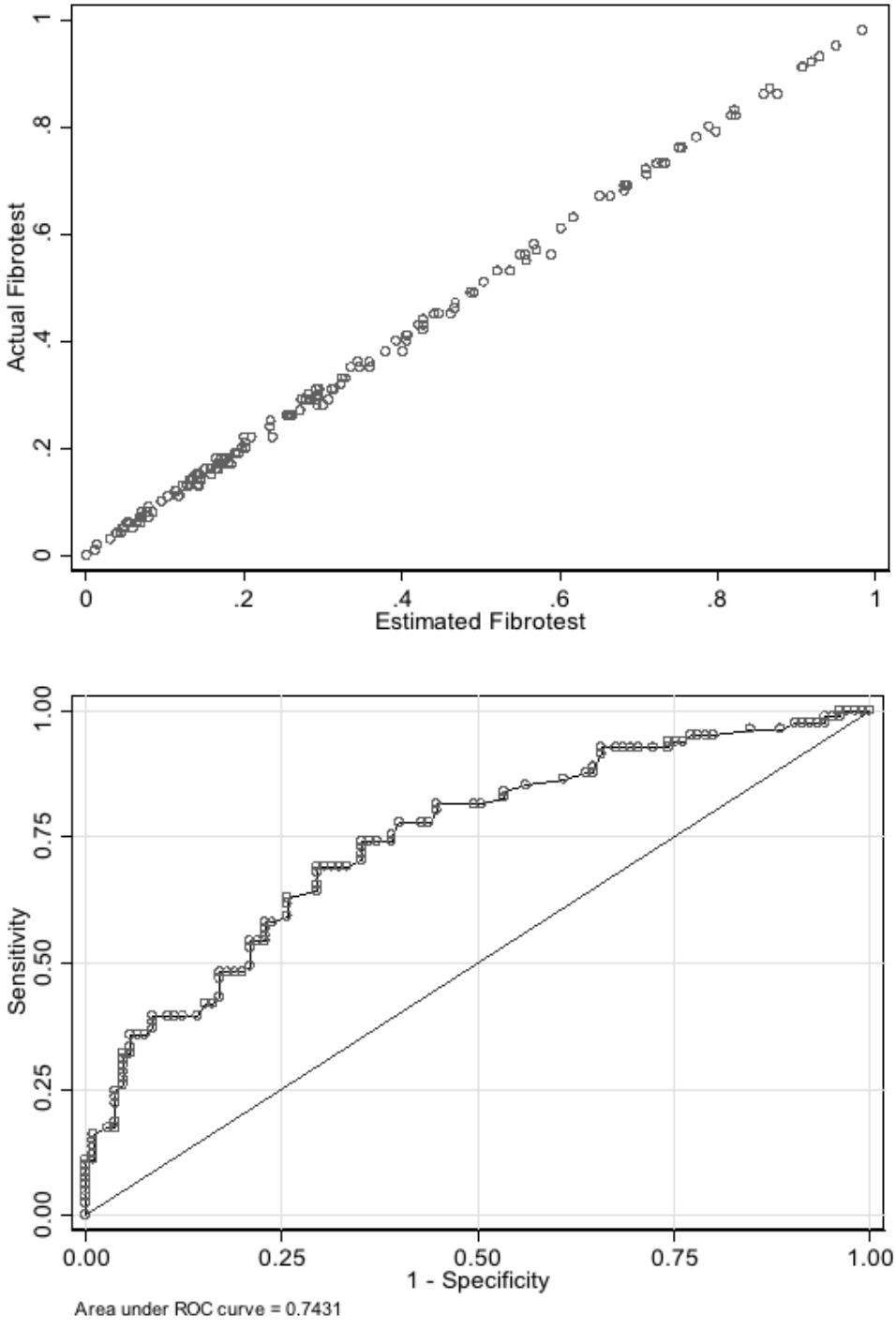
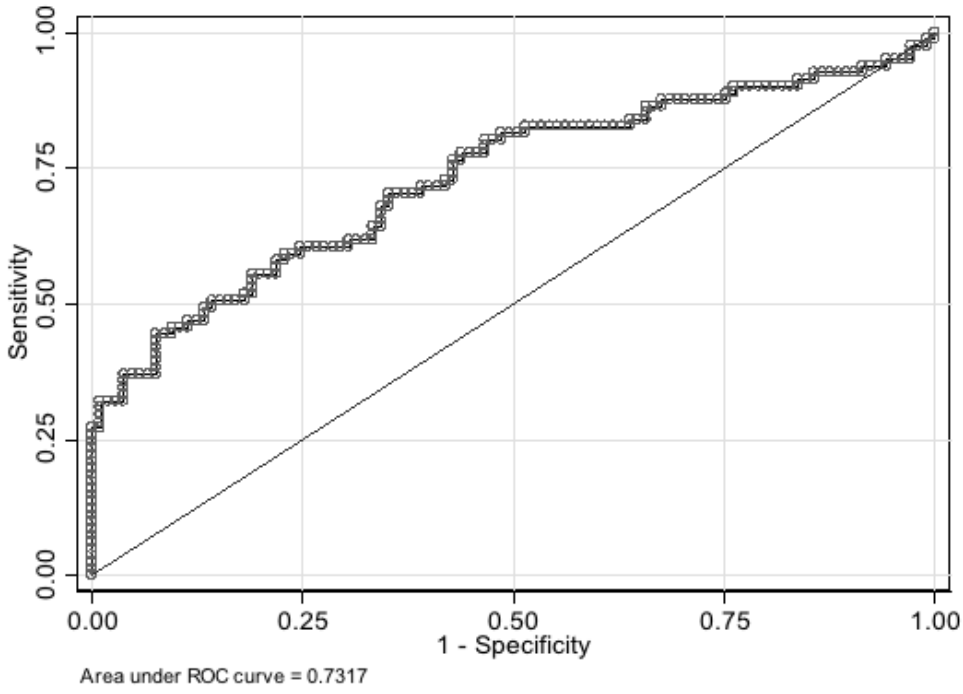


**Figure 3.** ROC (receiver operating characteristics) curves of a) Fibrotest and b) Forns scores.





# Chemokine receptor 5 polymorphism in myocardial infarction patients from Luxembourg

Sophie Rodius[a], Christine Lambert[b], Carole Devaux[b], Jean-Claude Schmit[b], Yvan Devaux[a,*], Daniel R. Wagner[a,c]

[a] *Laboratory of Cardiovascular Research, Centre de Recherche Public-Santé, Luxembourg*

[b] *Laboratory of Retrovirology, Centre de Recherche Public-Santé, Luxembourg*

[c] *Division of Cardiology, Centre Hospitalier, Luxembourg.*

** Corresponding author. Laboratory of Cardiovascular Research, Centre de Recherche Public Santé, 120 route d'Arlon, L1150 Luxembourg. Tel +352 26970300; Fax +352 26970396; E-mail: yvan.devaux@crp-sante.lu*

**Abstract**

**Background:** The CC-chemokine receptor 5 (CCR5) is regulating inflammatory pathways and may thus be implicated in the development and progression of heart failure (HF). A 32 base pair deletion of the ccr5 gene, called CCR5Δ32, prevents the expression of CCR5 at the cell surface. We analyzed the association between the CCR5Δ32 deletion and the risk and severity of myocardial infarction (MI) in a cohort of patients from Luxembourg.

**Methods:** Using TaqMan allelic discrimination assay, we genotyped a total of 1080 patients undergoing coronary angiography. This population contained 3 groups of patients: controls with atypical chest pain, abnormal stress testing but normal coronary angiography (n=154), patients with angina who underwent uncomplicated primary coronary intervention (n=230), and patients with acute MI (n=696). In MI patients, left ventricular ejection fraction (LVEF) was determined 1-month after MI with echocardiography.

**Results:** The frequency of the CCR5Δ32 deletion was 16.3% in the global population, and was similar between controls, patients with angina and MI patients. The deletion was not associated with variations of plasma levels of creatine phosphokinase and troponin T, nor it was associated with LVEF, New York Heart Association class or 2-year mortality. The frequency of the deletion was comparable between MI patients with LV dysfunction (EF≤40%, n=82) and no LV dysfunction (EF>40%, n=402).

**Conclusions:** The frequency of the CCR5Δ32 deletion in Luxembourg is similar to that observed in other European countries and is not associated with the risk of developing MI and LV dysfunction.

**Keywords:** CC-chemokine receptor 5; genetic polymorphism; myocardial infarction; ventricular dysfunction.

## 1. Introduction

Following myocardial infarction (MI), the left ventricle (LV) undergoes several changes in size, shape and function, all of them constituting the remodeling process. This process can be either beneficial, restituting the functional capacity of the heart, or deleterious, leading to LV enlargement, myocyte slippage and hypertrophy, ultimately culminating to the development of heart failure (HF) [1-3]. The early steps of LV remodeling are associated with an acute inflammatory response mediated mainly by infiltrated neutrophils and macrophages. Leukocytes recruited in the infarcted heart remove necrotic and apoptotic cells as well as matrix debris and secrete various growth factors, cytokines and chemokines that further modulate extra-cellular matrix synthesis, myofibroblasts proliferation and neoangiogenesis [4, 5].

Chemokines are crucial regulators of cardiac inflammation and LV remodeling since they orchestrate the migration of circulating leukocytes and other cell types and their recruitment into inflamed or injured tissues. They bind G-protein coupled cell surface receptors such as the CC-chemokine receptor CCR5, which is expressed on T-cells, macrophages, coronary endothelial cells and aortic smooth muscle cells [6-8]. CCR5 binds the chemokines RANTES (regulated on activation, normal T-cell expressed and secreted), MCP2 (macrophage/monocyte chemotactic protein 2), MIP-1α and MIP-1β (macrophage inflammatory proteins). The presence of these chemokines in atherosclerotic lesions [6, 9-12] suggests that CCR5 may play a role in atherosclerosis and therefore plaque stability.

Despite recent advances in reperfusion therapies, the occurrence of HF after MI remains associated with a more than 10-fold elevated risk of death [13]. Since HF is potentially preventable, the use of early prognostic biomarkers of HF may considerably decrease its incidence. In the search for new biomarkers of HF, identification of genetic risk markers, such as single nucleotide polymorphisms, has become a priority.

Among the several polymorphisms described in the CCR5 gene, the CCR5Δ32 deletion has been well documented since it highly enhances resistance to HIV-1 in homozygote patients, CCR5 acting as a co-receptor for HIV-1 [14, 15]. This 32bp deletion in CCR5 open reading frame results in a frame shift and premature translation termination, giving rise to a non-functional protein that is not expressed at the cell surface. Cardioprotective effect of the CCR5Δ32 deletion was suggested by a study in Spanish MI patients [16]. However, genetic studies performed thereafter provided conflicting results [17-20]. Interestingly, a recent study reported beneficial cardiovascular effects of the CCR5Δ32 mutation, as it decreases plasma triglycerides and increases high density lipoprotein cholesterol [21]. Therefore, the association between the CCR5Δ32 mutation and the risk and outcome after MI is still a matter of debate.

The purpose of the present study was to analyze the frequency of the CCR5Δ32 deletion in a population of cardiac patients from Luxembourg and to determine potential correlations with the risk of developing a MI and its outcome.

## 2. Materials and Methods

### 2.1. Patients

The population used in this study included patients enrolled in a national registry undergoing coronary angiography. The protocol has been approved by the local ethics committee and informed consent has been obtained from all subjects. This population included 3 groups of patients. First, normal controls with atypical chest pain, abnormal stress testing but normal coronary angiography. Second, patients with stable and unstable angina who underwent uncomplicated percutaneous coronary intervention (PCI). Cardiac enzymes were normal before and after the intervention. The third group included patients with acute MI treated with primary PCI. Acute MI was defined by the presence of chest pain, significant ST elevation, and increase in creatine phosphokinase (CPK) and troponin T (TnT) plasma levels to greater than 2-fold upper limit of normal. All MI patients underwent successful mechanical reperfusion and stenting of the infarct artery within 12 hours of onset of chest pain. Blood samples were obtained during mechanical reperfusion and LV function was determined by echocardiography 1-month after MI.

2.2. Genotyping

Genomic DNA was extracted from the buffy coat of centrifuged blood using FlexiGene kit 250 (Qiagen, Hilden, Germany) according to the manufacturer protocol. Detection of the CCR5 polymorphism was performed by TaqMan single nucleotide polymorphism allelic discrimination on a BioRad iQ5 apparatus using the following primers and probes: CCR5 forward primer: AAGGTCTTCATTACACCTGCAGC, CCR5 reverse primer: AGCAGCGGCAGGACCA, CCR5 wild-type probe: FAM-ACAGTCAGTATCAATTCTGGAAGAATTTCCAG-TAMRA, CCR5Δ32 probe: VIC-TCTCATTTTCCATACATTAAAGATAGTCATCTTGG-TAMRA. The 20μL PCR reaction mix contained 15ng of genomic DNA, 12.5μL of TaqMan Universal PCR Master Mix (Applied Biosystems), 1.1μL of each 20μmol/L primer and 0.3μL of each 15μmol/L probe. The PCR reaction was composed of 1 cycle at 50°C for 2min, followed by 1 cycle at 95°C for 10min and 40 cycles at 95°C for 15s and 62°C for 1min.

### 2.3. Statistical analysis

Two-tailed t-test and Mann-Whitney test were used to compare two groups of continuous data. Comparisons between multiple groups were achieved with one-way ANOVA. Kruskal-Wallis one-way ANOVA on ranks was performed for non-normally distributed data. Frequencies were compared with chi-square test with Yates correction for continuity. SigmaPlot 11.0 software was used for these analyses.

## 3. Results

### 3.1. Patient characteristics

Table 1 summarizes the clinical characteristics of the 1080 patients enrolled in this study and divided in 3 sub-groups: 154 patients with chest pain but normal coronary arteries (controls), 230 patients with stable and unstable angina undergoing uncomplicated PCI (angina), and 696 patients with acute MI undergoing primary PCI (MI). Gender and body mass index were similar between the 3 groups. MI patients were younger than controls and angina patients, were more often smokers, and had less hypercholesterolemia and hypertension. 2-year death rate was significantly higher in MI patients. Most MI patients presented a favorable outcome after infarction, as attested by the high frequency of patients with a New York Heart Association (NYHA) class 1 or 2 (90.9%) and the median LV ejection fraction (EF, 50%).

**Table 1. Clinical characteristics.**

| | Controls (n=154) | Angina (n=230) | MI (n=696) | *P* |
|---|---|---|---|---|
| **Age**, y (median-range) | 64 (22-87) | 68 (37-87) | 60 (24-91) | <0.001 |
| **Male gender** % | 65.6 | 76 | 75.9 | 0.57 |
| **Body Mass Index** (median-range) | 27 (16-35) | 27,3 (17-43) | 27 (18-56) | 0.78 |
| **Smoking** % | 32.5 | 32.6 | 45.1 | 0.03 |
| **Diabetes** % | 16.9 | 27 | 21.3 | 0.15 |
| **Hypercholesterolemia** % | 45.4 | 56.1 | 41.7 | 0.07 |
| **Hypertension** % | 57.1 | 60.9 | 43.8 | 0.02 |
| **CPK**, units (median-range) | - | - | 1316 (34-11392) | |
| **TnT**, ng/mL (median-range) | - | - | 3,45 (0.01-31.64) | |
| **EF**, % (median-range) | - | - | 50 (15-89) | |
| **NYHA** % **class 1** | - | - | 63.2 | |
| **class 2** | - | - | 27.7 | |
| **class 3** | - | - | 7.8 | |
| **class 4** | - | - | 1.3 | |
| **Death** % | 1.4 | 3.9 | 7.3 | 0.02 |

*Controls: patients with chest pain but normal coronary arteries. Angina: patients with stable or unstable angina undergoing uncomplicated primary coronary intervention; MI: patients with acute myocardial infarction undergoing primary primary coronary intervention. CPK: creatine phosphokinase; TnT: troponin T; EF: ejection fraction; NYHA: New York Heart Association class. EF was available for 484 MI patients; NYHA was available for 617 MI patients and 2-year mortality was available for 139 controls, 208 angina and 633 MI patients.*

### 3.2. Distribution of the CCR5Δ32 deletion in the global population

The frequency of the CCR5Δ32 polymorphism in the whole population was determined by allelic discrimination using TaqMan assay. Results of the genotyping presented in Fig. 1A indicate that 16.3% of the patients carried the deletion on at least one allele, the frequency of mutant homozygote patients being low (6 patients, representing 0.6% of the entire population).

### 3.3. Relationship between the CCR5Δ32 deletion and the risk of developing MI

In order to bring to the front a potential correlation between the CCR5Δ32 deletion and the occurrence of acute MI, we compared the frequency of this deletion between 3 groups of patients, namely controls, patients with angina and patients with MI. Results shown in Fig. 1B indicate that the frequency of the CCR5Δ32 deletion does not differ between the 3 groups, ranging between 13.6% and 17.8% (P=0.84). Additional analyses revealed that the CCR5Δ32 deletion was not associated with CPK and TnT plasma levels (P=0.50 and P=0.75, respectively, Fig. 2). These data show that the CCR5Δ32 deletion is not associated with the risk of developing MI and infarct severity.

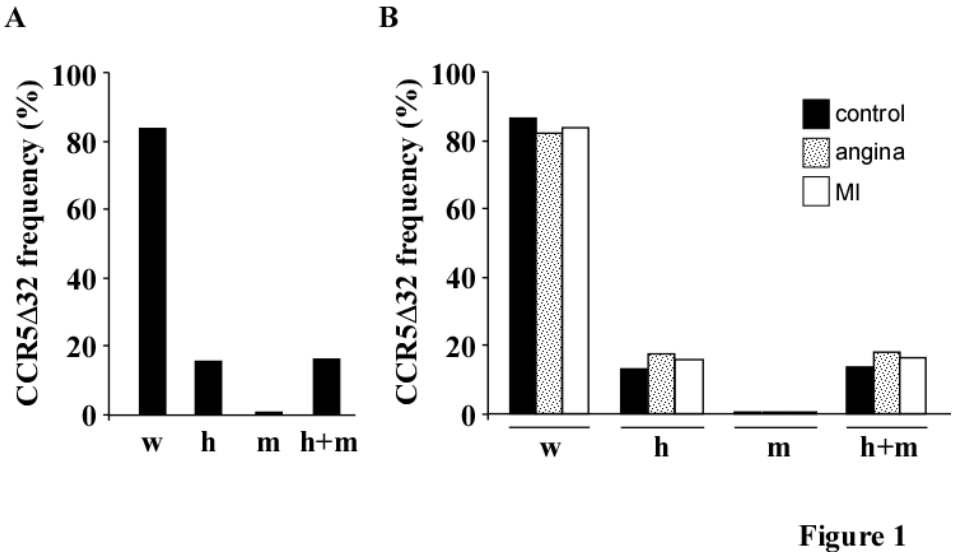



Figure 1

**Fig. 1.** *Frequency of the CCR5Δ32 deletion in the global population (A) and in the 3 sub-groups of controls (n=154), angina (n=230) and MI (n=696) patients (B). w: wild-type; h: heterozygote; m: homozygote mutant. Frequencies were compared using Chi-square test (P<0.001).*

### 3.4. Association between the CCR5Δ32 deletion and outcome after MI

To evaluate the association of the mutation with clinical outcome after MI, we analyzed the association of the deletion with 3 different end points: mortality, NYHA class and EF measured 1-month after MI. 2-year death rate was close to 7% in wild-type and mutated patients, with no significant differences (P=0.86). Also, no significant difference was observed for NYHA class (P=0.97, Table 2). Median 1-month EF varied from 48% to 50% between wild-type, heterozygote and mutant homozygote patients (P=0.63, Fig. 3). Therefore, the CCR5Δ32 deletion is not associated with a specific outcome after MI.

**Table 2. Association between the CCR5Δ32 deletion and outcome in 696 MI patients.**

| | | w | h | m | h+m |
|---|---|---|---|---|---|
| **Death** | **n** | 39 | 7 | 0 | 7 |
| | **%** | 7.4 | 7 | 0 | 6.7 |
| **NYHA 1** | **n** | 322 | 63 | 3 | 66 |
| | **%** | 62.9 | 64.3 | 75 | 64.7 |
| **NYHA 2** | **n** | 145 | 25 | 1 | 26 |
| | **%** | 28.3 | 25.5 | 25 | 25.5 |
| **NYHA 3** | **n** | 39 | 8 | 0 | 8 |
| | **%** | 7.6 | 8.2 | 0 | 7.8 |
| **NYHA 4** | **n** | 6 | 2 | 0 | 2 |
| | **%** | 1.2 | 2 | 0 | 2 |

*w: wild-type; h: heterozygote; m: homozygote mutant. NYHA: New York Heart Association class. 2-year mortality was available for 526 wild-type, 100 heterozygote and 4 homozygote mutant. NYHA was available for 512 wild-type, 98 heterozygote mutant and 4 homozygote mutant.*

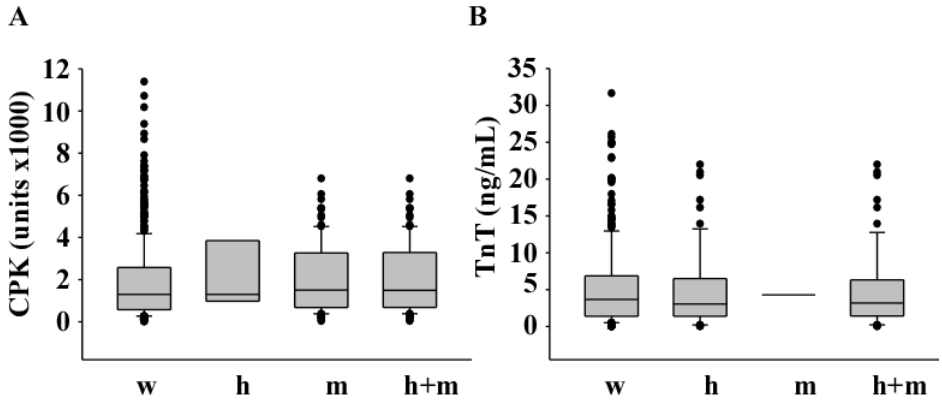



**Figure 2**

**Fig. 2.** *Association between the CCR5Δ32 deletion and infarct severity. Box-plot representing CPK (A) and TnT (B) plasma levels in wild-type (w), heterozygote (h) and homozygote mutant (m) patients. No statistical significance was observed (CPK: P=0.50, TnT: P=0.75).*

### 3.5. Correlation between the CCR5Δ32 deletion and LV remodeling

To investigate a potential correlation between the CCR5Δ32 polymorphism and LV remodeling after MI, we assessed the frequency of the mutation in 2 groups of MI patients: patients without LV dysfunction after MI (EF>40%, n=402) and patients with LV dysfunction after MI (EF≤40%, n=82). Patients without LV dysfunction had a lower 2-year mortality rate (P<0.001), were more often on NYHA

class 1-2 (P=0.002), and had higher EF (P<0.001) than patients with LV dysfunction (Table 3). They also were younger, and had lower CPK and TnT levels (Table 3). Fig. 4 represents the proportion of wild-type, heterozygote and mutant homozygote individuals in the 2 groups. The frequency of the CCR5Δ32 deletion was similar between patients with and without LV dysfunction (P=0.60), indicating that the deletion is not correlated with LV remodeling after MI.

**Table 3. Clinical characteristics of 2 groups of MI patients.**

| | EF≤40 (n=82) | EF>40 (n=402) | *P* |
|---|---|---|---|
| **Age**, y (median-range) | 65 (32-91) | 59 (29-90) | 0.003 |
| **Male gender** % | 80.5 | 77.4 | 0.9 |
| **Body Mass Index** (median-range) | 26.9 (20-38) | 27 (18-47) | 0.11 |
| **Smoking** % | 40.2 | 45.5 | 0.66 |
| **Diabetes** % | 28 | 20 | 0.25 |
| **Hypercholesterolemia** % | 34.1 | 45 | 0.29 |
| **Hypertension** % | 43.9 | 41 | 0.85 |
| **CPK**, units (median-range) | 2446 (34-9383) | 1241 (36-10717) | <0.001 |
| **TnT**, ng/mL (median-range) | 5.4 (0.02-26.1) | 3.5 (0.01-31.64) | 0.009 |
| **EF**, % (median-range) | 35 (15-40) | 52.5 (41-89) | <0,001 |
| **NYHA** % **class 1** | 47.8 | 62.9 | 0.002 |
| **class 2** | 28.3 | 29.2 | |
| **class 3** | 20.9 | 6.5 | |
| **class 4** | 3 | 1.4 | |
| **Death** % | 17.6 | 5.5 | <0.001 |

*One group of patients had LV dysfunction with altered EF (≤40%) and the other group had no LV dysfunction with preserved EF (>40%). CPK: creatine phosphokinase; TnT: troponin T; EF: ejection fraction; NYHA: New York Heart Association class. NYHA was available for 67 patients with EF≤40% and 367 with EF>40%. 2-year mortality was available for 74 patients with EF≤40% and 363 patients with EF>40%.*

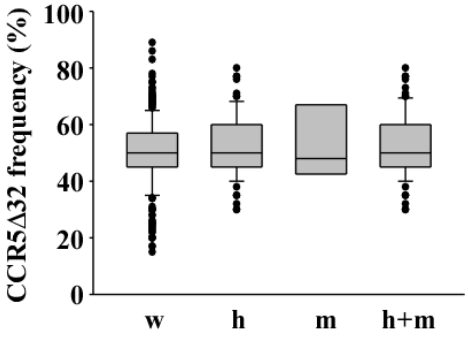



Figure 3

**Fig. 3.** *Association between the CCR5Δ32 deletion and 1-month EF. w: wild-type, h: heterozygote and m: mutant homozygote patients. No statistical significance was detected (P=0.63).*

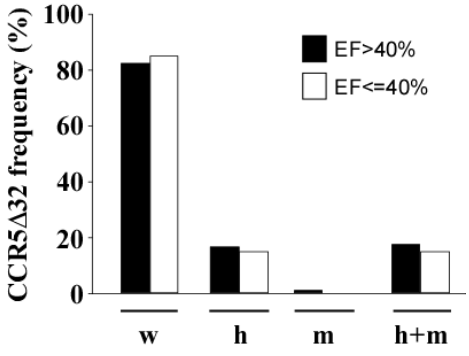



Figure 4

**Fig. 4.** *CCR5Δ32 deletion frequency in 2 groups of MI patients, on the one hand patients without LV dysfunction after MI (EF>40%, n=402) and on the other hand patients with LV dysfunction after MI (EF≤40%, n=82). w: wild-type, h: heterozygote, m: mutant homozygote. The frequency of CCR5Δ32 deletion did not differ between the 2 groups (P=0.60).*

## 4. Discussion

The chemokine receptor CCR5 is functionally implicated in the regulation of inflammation and may therefore affect the course of LV remodeling after infarction. The main purpose of this study was to characterize the frequency of the CCR5Δ32 deletion in a population of cardiac patients in order to assess a potential correlation between the deletion, the risk of developing MI and its clinical outcome. No significant association between the presence of the CCR5Δ32 deletion, occurrence of MI, infarct size, clinical outcome and LV dysfunction was found in the population studied.

Genotyping of a cohort of 1080 patients from Luxembourg revealed a frequency of 16.3% for the CCR5Δ32 deletion. This result is comparable to previous studies indicating that the allele frequency of the mutation presents a north-south cline in Europe with higher frequencies (16%) in the north [22].

In order to assess a potential correlation between the CCR5Δ32 deletion and the risk of developing MI, we studied the frequency of this deletion in 3 different populations: patients with chest pain but normal coronary arteries, patients with angina undergoing uncomplicated PCI and patients with acute MI undergoing primary PCI. As the CCR5 receptor is expressed on immune cells as well as coronary endothelial cells and aortic smooth muscle cells [6-8], mutations in the CCR5 gene, leading to absence or even decrease of CCR5 protein expression, may affect inflammation and MI. However, while a study performed on a Spanish population reported a lower frequency of the CCR5Δ32 mutation in MI patients compared to controls, and therefore a cardioprotective effect of the deletion [16], others failed to show an association with the risk of developing coronary artery diseases [17-20]. In the present study, the CCR5Δ32 deletion was not associated with the risk of developing MI, neither with infarct severity as assessed by CPK and TnT plasma levels, nor with clinical outcome after MI, as indicated by the NYHA class, 2-year mortality rate and 1-month LVEF.

To assess a potential correlation between the CCR5Δ32 deletion and LV remodeling, we divided the group of 696 MI patients into two groups: on the one hand

patients who developed LV dysfunction (EF≤40%, unfavorable outcome) and on the other hand patients who did not (EF>40%, favorable outcome). Despite significant differences between the 2 groups of patients, low EF patients being older, having more often a prior MI, larger infarct and worse clinical outcome, the frequency of the CCR5Δ32 deletion was not significantly different between both groups. Therefore, our data show for the first time that the CCR5Δ32 deletion is not associated with LV remodeling and dysfunction after MI.

In conclusion, we report that the frequency of CCR5Δ32 deletion is not associated with the risk of developing MI and subsequent LV dysfunction. This study is limited by the relatively low number of patients included, and consecutively the very low number of homozygote mutant patients. This limitation applies to most of the genetic studies on CCR5Δ32 deletion. Our results suggest that the CCR5 receptor may not be functionally implicated in the development of MI and HF.

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# Predictive Relevance of Clinical Scores and Inflammatory Parameters in Secondary Peritonitis.

*Nikolaus P. Zügel[1,2], MD; Martin Kox[1], MD; Michael Lichtwark-Aschoff[4], MD; Cornelia Gippner-Steppert[5], PhD; Marianne Jochum[5], PhD*

*[1]Department of General and Visceral Surgery, Centre Hospitalier Emile Mayrisch Luxembourg: PD Dr. N. Zügel, MD, Assoc Prof. of Surgery*
*[2]Department of Surgery, Klinikum Grosshadern LMU München: Prof. KW. Jauch, MD*
*[3]Department of Anaestiology, Central Hospital Augsburg: Prof. H. Forst, MD*
*[4]Department of Clinical Chemistry and Clinical Biochemistry, Klinikum Innenstadt LMU Munich: Prof. M. Jochum, PhD*

***Corresponding author: Dr. N.P. Zügel, MD, Assoc Prof. of Surgery***

*General and Visceral Surgery Unit, Centre Hospitalier Emile Mayrisch, Rue Emile Mayrisch; L-4005 Esch-sur-Alzette, Luxembourg.*
*Telephone: 00352-571199318; Fax: 00352-540217; e-mail: nikolaus.zuegel@chem.lu*

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**Hypothesis:** To measure and evaluate clinical scores and various inflammation parameters for providing a better outcome assessment of patients with secondary peritonitis.

**Design:** Prospective study.

**Setting:** ICU of a university and a university affiliated hospital.

**Patients:** Fifty-six patients with severe secondary peritonitis were enrolled in this study executed within 4 years.

**Measurements and Main Results:** Blood samples were taken preoperatively and 2, 6, 8, 12, 18, 24, 30, 36, 42 and 48 hours post operation, thereafter every 12th hour until day 5 respectively once daily until day 14. Etiology of peritonitis, clinical score systems (APACHE II, MOF and SOFA), and 27 mainly with activity tests or enzyme-immunoassays measurable inflammation parameters were simultaneously analyzed and stratified into lethal outcome (n=11) or survival (n=45), respectively. The etiological distribution of peritonitis was identical among both groups. Proportion of intraperitoneal fungi, E. coli, and bacteroids was substantially higher during the primary operation in the group with lethal outcome. With increasing significance initial and follow-up APACHE II, MOF and SOFA scores provided higher values in this group. Various plasma/serum parameters of hemostasis, leukocyte proteolytic system, acute phase reaction, cytokine system, cell adhesion, opsonization, and main organ functions showed significantly different values between both groups at the preoperative stage and/or during observation period I (day 0-4). Logistic regression analysis revealed the SOFA score and neopterin concentration as the combination with the best sensi-

tivity (63.6 %) and specificity (93.2 %) for predicting the patients' survival even at the preoperative stage. For the observation period I, the combination of SOFA score and TNF receptor II showed the highest predictive sensitivity (72.7 %) and specificity (95.6 %).

Conclusion: Evaluation of the severity of secondary peritonitis using a scoring system with high prognostic relevance could conceivably result in an earlier and adequate application of intensive care such as hemofiltration, administration of immunoglobulins and serial abdominal lavage to improve successful outcome.

**Key Words:** Secondary peritonitis–lethal multiple organ failure–predictive value–APACHE-II, MOF, SOFA score–neopterin–TNF receptor II–hemostasis–cytokine system–cell adhesion molecules–opsonization

In Memoriam: Prof. Jens Witte †12.06.2003

## INTRODUCTION

Risk assessment of patients with secondary peritonitis is usually performed using general classifications such as those of the American Society of Anesthesiology (ASA) (1). According to these criteria, patients suffering from severe peritonitis with or without serious underlying disorders belong to class IV and V (inactive patient, with life-threatening system disorder). Consequently, the operation risk is high (2), but an individual risk assessment is not possible because all the patients in fact belong to the high-risk group. Nevertheless, preoperative co-morbidity in peritonitis patients correlates with the incidence of postoperative morbidity and mortality. Largely irrespective of the operation, the postoperative morbidity and mortality risk increases with age (3, 4). Therefore, assessing the severity of the disorder using a combination of clinical scores and systemically measurable inflammation parameters with the highest predictive value could guide the application of intensive care measures such as hemofiltration, administration of immunoglobulins, and serial abdominal lavage with better chances for successful outcome.

To prove this assumption, numerous clinical and biochemical parameters can be monitored at the beginning and during the further course of the disease. The initial graduation of peritonitis is accessible by the «Mannheimer-Peritonitis-Index» (MPI) according to Lindner et al. (5). In addition, the clinical scores APACHE II («Acute Physiology And Health Evaluation»), MOF («Multiple Organ Failure»), and SOFA («Sepsis-related Organ Failure Assessment») are applied usually to judge the severity of the pathophysiological inflammatory condition. In this respect, the APACHE II score comprises a great variety of physiological parameters besides age and associated disease criteria (6). An approximate quantification of the severity of MOF is achievable by the classification suggested by Goris et al. (7), whereas a more detailed judgement of septic organ dysfunctions was provided by Vincent et al. (8) with the so-called SOFA score. The usefulness of this scoring

system in intensive care was just recently confirmed by Moreno et al. (9). Moreover, parameters of coagulation such as thrombocytes, partial thromboplastin time (PTT), prothrombin time (Quick) or latent thrombin activity (prothrombin) and thrombomodulin (10) as well as the acute phase protein CRP (C-reactive protein) and total leukocyte count serve as well-known markers of a general inflammatory response. Regarding the destructive proteolytic potency of the leukocyte proteinases PMN-elastase and cathepsin B (11,12) measurement of these rapidly released cellular inflammatory agents should be also of high value in judging severity and outcome of peritonitis. As claimed recently, procalcitonin (PCT), a marker of bacterial sepsis may act specifically as a mediator of inflammatory response to infection and thus influence outcome of peritonitis patients (13-16).

Furthermore, parameters of the cytokine system like interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin-1 receptor antagonist (IL-1ra) or tumor necrosis factor receptor II (TNF-RII) in addition to the soluble adhesion molecules P-selectin, E-selectin, L-selectin, and intercellular adhesion molecule 1 (ICAM-1) are used increasingly for prognosis of patients suffering from acute inflammation (17-22). In the setting of peritonitis early disturbance of opsonization capacity (OC) attributed to complement C3, immunoglobulin IgG and probably also to fibronectin seems to play a major role in the development of forthcoming reversible or lethal organ failure (23). Thus, measuring OC may be of special predictive relevance in these patients. Last not least, procollagen-III-peptide (P-III-P) as an indicator of enhanced fibroblast activity and/or reduced elimination capacity of liver cells (24, 25) as well as neopterin as a marker of activated monocytes/macrophages and/or reduced kidney eliminatory function (26, 27) may provide further prediction of the patients' outcome.

Summarizing the above mentioned studies and many others as well, one has to take into account that the prognostic value of the clinical scores and numerous inflammation indicators was examined mostly separately for each parameter in different clinical settings so far. Therefore, the aim of our study was to evaluate for the first time in a close-meshed monitoring approach the predictive meaningfulness of the clinical score systems MPI, APACHE II, MOF, and SOFA simultaneously with a wide variety of inflammation parameters indicating the development of reversible or lethal multiple organ failure in patients with secondary peritonitis. In addition, we tried to establish a reliable combination comprising only the most predictive clinical and biochemical parameters which may allow application of such a scoring system also in the more restrictive routine handling of peritonitis patients.

## Materials and Methods

*Patients and Treatment*. After approval by the local ethic committees of the participating hospitals and informed consent 56 patients (n=36 LMU Munich, n=20 Klinikum Augsburg, Germany) were included in a prospective study performed

over a period of 4 years according to the following entry criteria: local bacterial infection and fibrin deposits in two or more quadrants, body temperature >38 °C, leukocytes >10 000/µl or <3 000/µl, age >18 years. The patients were treated according to the guidelines for peritonitis care. Following primary intervention with focal sanitation and subsequent lavage, patients' treatment was continued on surgical ICU including monitoring, mechanical ventilation (if required) and circulatory support (dopamine 14 µg/kg BW/min, noradrenalin and dobutamine depending on the clinical situation). Sedation was achieved with fentanyl and midazolam; ranitidin was given to prevent stress ulcer, and intravenous high molecular sodium heparin (4 IU/kg BW) was administered routinely. The latter medication was started 6 hours postoperatively. The initial antibiotic therapy consisted of a combination of a 2nd generation cephalosporin and metronidazol. Selective intestinal decontamination was performed in all patients. Blood components were substituted specifically in case of clotting disturbance prefering fresh frozen plasma (FFP) to individual coagulation factors or mixtures (e.g. PPSB). Platelets were supplemented as indicated in case of hemorrhage due to thrombocytopenia (<80 000/µl). Erythrocyte concentrates were applied in cardiovascular instability and severe hypovolemia. The patients were nourished parenterally with glucose/amino acid solutions and fatty acids. A change to enteral diet was carried out after the 5th day or after the onset of intestinal activity.

*Data Collection Protocol*. Data were collected over the first postoperative 14 days or until discharge from the ICU. Clinical and routine laboratory parameters were monitored immediately before and after the operation (2 hours after abdominal wall closure) as well as daily at 8 a.m. during the further observation period. Arterial blood samples for estimation of special inflammation parameters were taken preoperatively and 2, 6, 8, 12, 18, 24, 30, 36, 42 and 48 hours post operation, thereafter every 12th hour until day 5 respectively once daily until day 14. The blood samples were processed to citrated plasma or serum and centrifuged at 4,000 rpm for 15 minutes at 10° C. The supernatants were deep-frozen in aliquots at -70° C until assayed.

*Analysis of Peritonitis Severity, Organ Failure, and General Laboratory Parameters*. Initial severity of peritonitis was judged according to the Mannheimer-Peritonitis-Index (MPI) (5). Reduction and/or persistence of organ dysfunctions were determined using the APACHE II (Acute Physiology And Health Evaluation), MOF (Multiple Organ Failure) and the SOFA (Sepsis-related Organ Failure Assessment) scores (6, 7, 8). General laboratory parameters such as $pO_2/FiO_2$ quotient, leukocyte and thrombocyte count, bilirubin, creatinine, total serum protein, partial thromboplastin time (PTT), and prothrombin time (Quick) were determined employing routine methods.

*Analysis of Special Inflammation Parameters.* Latent thrombin activity (*prothrombin*) was measured in citrated plasma (standard range: 80 - 125 %) using a specific chromogenic substrate test (S-2238, Haemachrom Diagnostica, Ger-

many). The quantitative determination of soluble *thrombomodulin* (plasma standard range: 16.8–42.6 ng/ml) was achieved with a sandwich-ELISA from Diagnostica Stago, France.

The neutrophil proteinase *PMN*-elastase (plasma standard range: 80–120 ng/ml) in complex with $\alpha_1$ proteinase inhibitor ($\alpha_1$ PI) was measured with a modified version of a commercially available two-site sandwich-ELISA (E. Merck, Darmstadt, Germany). The proteolytic monocyte enzyme cathepsin B (plasma standard range: 60–120 mU/l) was determined with a fluorimetric test according to Assfalg-Machleidt et al. (28). The most important acute phase protein C-reactive protein (CRP, plasma standard range: <0.5 mg/dl) was determined with radial immunodiffusion (RID) using LC partigen plates (Behringwerke, Marburg, Germany). The infection-associated procalcitonin (PCT, plasma standard range: < 0.5 µg/l) was quantified with an immuno-luminometric assay (ILMA) from Brahms Diagnostica GmbH, Berlin, Germany.

*Interleukin-1ra* (*IL-1ra,* serum standard range: 157 - 3170 pg/ml) was determined quantitatively using a sandwich-ELISA from Amersham, Braunschweig, Germany. *Interleukin-6* (*IL-6*, serum standard range: 0 - 6 pg/ml) and *interleukin-8* (*IL-8,* serum standard range: 0 - 30 pg/ml) were quantified with sandwich-ELISAs from R & D Systems, Abingdon, United Kingdom. Soluble *tumor necrosis factor receptor II* (*TNF-RII; 80 kDa,* serum standard range: 3.4 - 10.8 ng/ml) was quantified with a sandwich-ELISA from Bender MedSystems, Vienna, Austria).

Concentrations of the following soluble adhesion molecules in serum were quantified with specific sandwich-ELISAs from Bender MedSystems, Vienna, Austria: L-Selectin (standard range: 487 - 1096,3 ng/ml), E-selectin (standard range: 15 - 25 ng/ml), P-selectin (standard range: 111 - 266 ng/ml) and ICAM-1 (standard range: 180 - 280 ng/ml).

The opsonins *immunoglobulin G* (*IgG,* plasma standard range: 800 - 1800 mg/dl), *complement C3* (plasma standard range: 56 - 120 mg/dl) and *fibronectin* (plasma standard range: 25 - 40 mg/dl) were determined with RID using LC partigen plates (Behringwerke, Marburg, Germany). The *opsonization capacity* (mainly comprising IgG and C3) was detected by a chemiluminescence measurement (modified according to *Billing et al.* (23)) in highly diluted normal donor blood after activation of the phagocytes with zymosan which was preopsonized with patient serum. The activity was expressed in percent of a standard serum.

For the quantitative determination of *procollagen-III-peptide* (*P-III-P,* plasma standard range: 0.3 - 0.8 E/ml) a radio-immunoassay (RIA) was applied using the principle of a 2-stage sandwich test (CIS Diagnostik GmbH, Dreieich, Germany). *Neopterin* concentration (serum standard range: 1 - 10 nmol/l) was determined with a RIA from Henning, Berlin, Germany.

*Statistical Analysis.* Demographic data, clinical score systems and inflammation parameters were analyzed using SPSS. For statistical reasons (group size ³6) descriptive data evaluation shown in the figures was performed only for the first

eight postoperative days. Moreover, since a temporal kinetic effect was observed for most of the parameters, in particular for the indicators of the systemic inflammation response, a comparison of mean value (Wilcoxon-U-test, $p \leq 0.05$) was made between the groups (survivors/non-survivors) for the preoperative value and the observation segment I (day 0 to 4). Data are presented as mean ± SEM. logistical regression analysis (29, 30) was used to determine the factors with the highest specificity and sensitivity to predict survival of secondary peritonitis. Included into the calculation were both, the preoperative value at the time of the primary intervention and the mean value of observation segment I of each patient. Receiver operating characteristic (ROC-) curves were constructed and a test of the difference between areas under the ROC-curve was applied using the trapezoidal rule to approximate areas, which serves as a conservative estimate for the SDs and Kendall`s t as a measure of the correlation between the areas (31). The area under the ROC-curve reflects predictive value of any biochemical parameter or score system. A minimum rate of 0.8 is required to allow significant prognostic prediction.

Statistical advice and review of results was provided by Dr. M. Wiseman, Leibnitz-Rechenzentrum of the LMU Munich.

## Results

*Clinical Characteristics.* Eleven of the prospectively enrolled 56 patients (20 %) died as a consequence of multiple organ failure. Five of these patients succumbed within the first 4 days, two patients until day 10 and four patients between day 12 and 20 after enclosure to the study. A tendency towards a lower death rate in females (13 %) compared to males (27 %) became obvious.

The initial demographic data of the total group showed no significant differences between survivors and non-survivors (Table 1). Similarly the origins of peritonitis were evenly distributed between both groups (Table 2). Yet, the proportion of intraperitoneal fungi, E. coli, and bacteroids being detectable during the initial operation was already substantially higher in non-survivors (Table 3). The average lavage frequency among the groups did not differ significantly (non-survivors: 2.1 (± 0.6) vs. survivors: 2.4 (± 0.3), p=0.68).

*Clinical Score Systems*. As shown in Table 4 the mean intraoperative MPI of both patient collectives did not differentiate between later lethal outcome or survival. In contrast, at the preoperative stage (Table 4) and during the first 4 postoperative days (Table 5), the APACHE II, MOF and SOFA scores differed significantly between non-survivors and survivors showing significantly higher levels in patients with lethal outcome (Fig. 1). ROC-analysis demonstrated the superiority of the SOFA score both pre- and postoperatively for assessment of outcome (Fig. 2 and 3).

*Coagulation Parameters* (Fig. 4; Tables 6 and 7). Both at the time of entry to the study and during observation period I, the thrombocyte count in the blood showed

significantly higher values in survivors. With respect to plasmatic coagulation, the first and all follow-up values of PTT were significantly shorter in this patient collective compared to the lethal outcome group. Moreover, a higher prothrombin time (Quick) was noted initially and up to the 4th day concomitant with a higher latent thrombin activity (prothrombin) in the group of the survivors. In contrast, thrombomodulin showed a highly significant pathological increase even at the preoperative stage prior to the first intervention in the group of non-survivors compared with survivors. This difference persisted throughout the observation period I as well.

*Leukocyte Count, Proteinases and Acute Phase Proteins* (Fig. 5, Tables 6 and 7). With the exception of procalcitonin, all other inflammation parameters (leukocyte count, elastase, cathepsin B, CRP) in the preoperative blood/plasma samples showed no significant differences among the deceasing and the surviving patients. Under a temporally differentiated consideration of the mean value comparison of independent random samples, the groups demonstrated significantly different values for procalcitonin and cathepsin B within the first 4 days of observation (Table 7).

*Parameters of the Cytokine System* (Fig. 6, Tables 6 and 7). Despite comparably highly elevated initial values, the follow-up data of IL-1ra in non-survivors versus survivors were significantly different only during the first postoperative day showing an obvious delay in the decrease to the standard range in the lethal outcome group. With respect to IL-6 a similar systemic concentration pattern became obvious. Yet, due to the high standard deviation of the individual values, significant differences could be calculated only after the first postoperative day. In contrast to IL-1ra and IL-6, the mean value comparisons of IL-8 and TNF-RII differed significantly not only before the operation but also during the time segment I exhibiting significantly higher values in the non-survivors.

*Parameters of Cell Adhesion*. As depicted in Fig. 7, none of the soluble adhesion molecules provided significantly different values between survivors and non-survivors, neither initially, nor throughout the follow-up. For this reason, tabular overviews of the mean value comparisons with details of significance data were waived. Strikingly, however, soluble L-selectin and ICAM-1 exhibited consistently, yet there are not significantly higher circulating levels in survivors.

*Parameters of the Opsonization* (Tables 6 and 7). Although the immunological amount of the opsonic factor complement C3 and the opsonization capacity comprising mainly functional C3 and IgG showed obviously higher values in survivors, significant group differences could be seen only in the preoperative and very early postoperative stage. This difference persisted during observation period I only for C3, whereas the opsonins IgG and fibronectin showed no significant group differences.

*Parameters of Various Organ Functions* (Fig. 8, Tables 6 and 7). Throughout the whole observation period the parameters of renal function (serum creatinine,

neopterin) and of the pulmonary gas exchange (as indicated by the $pO_2/FiO_2$ quotient) showed significantly lower respectively higher values as an indication of the better clinical situation in patients overcoming the secondary peritonitis. P-III-P, a measure of the fibrotic transformation of liver tissue and/or of the disturbed eliminatory function of the liver, revealed a significantly better organ function, i.e. lower levels in survivors from the early observation time onwards. Although correlating with the P-III-P pattern, bilirubin, the common indicator of the liver elimination capacity, showed significant group differences only at the postoperative stage for the time segment day 0-4.

*Combination of Clinical Score Systems and Significant Inflammation Parameters.* As outlined above and shown in detail in Tables 6 and 7 significant differences between survivors and non-survivors occurred already at the preoperative stage with respect to the clinical score systems APACHE II, MOF and SOFA as well as to systemically measurable clinical-chemical routine parameters and more recent inflammation factors such as thrombocytes, PTT, thrombomodulin, procalcitonin, IL-8, TNF-RII, C3, opsonization capacity, creatinine*, neopterin*, pO2/FiO2 quotient and P-III-P. During the observation period I (mean value for day 0 to 4) not only the score systems APACHE II, MOdF* and SOFA* but also inflammation associated parameters like thrombocytes, PTT*, Quick, thrombomodulin, cathepsin B, procalcitonin, IL-8*, TNF-RII*, C3, creatinine*, neopterin*, pO2/FiO2 quotient, bilirubin, and P-III-P differed significantly between survivors and non-survivors (Wilcoxon-U-test: $p \leq 0.05$; * $p \leq 0.001$).

However, due to logistic and financial reasons it is impossible to measure all of the above mentioned significant outcome predictors during routine handling of peritonitis patients. Therefore, logistical regression analysis was applied to determine the most important preoperative factors with the greatest sensitivity and specificity regarding outcome. The combination of SOFA score (p=0.01) and neopterin serum concentration (p=0.03) at the preoperative stage had a sensitivity regarding nonsurvival of 64 % and positive preictive value (PPV) of 70%. The corresponding value for survival (specificity) amounted to 93 % and negative predictive value (NPV) to 91%.

To compute the probability of surviving secondary peritonitis in future studies (Table 8) the following equation can be applied:

$$\mathbf{R = 1/(1 + e^{-4.81 + 0.02 \times [neopterin] + 0.44 \times SOFA\ score})}$$

Taking the mean values of day 0 to 4 into accont the combination of SOFA score (p=0.007) and TNF-RII (p=0.08) level provides the most accurate prediction of death from peritonitis (sensitivity: 73 %; specificity: 96 %; PPV: 80%; NPV: 93%). Interestingly, calculating the prognosis from the values of a single daily morning sample rendered similar results as achieved when including the values of more than one sample per day throughout the observation period I.

Since it is self-evident that irrespective of the initial results an optimized intensive therapy ought to be carried out during observation period I (day 0-4) after primary focal sanitation, the prediction of outcome respectively of the therapeutic success may be evaluated with the following equation:

$$R_I = 1/(1 + e^{-6.49 + 0.06 \times [TNF\ R_{II}] + 0.64 \times SOFA\ score})$$

If $R_I$ approaches 1, the probability of surviving the peritonitis will equal 100 % (Table 9). If the computed value approaches 0, the further intensification of the therapy is most likely to be unsuccessful.

## Discussion

Postoperative morbidity and mortality of surgical patients increases with advancing age, largely irrespective of the operation procedure (3, 4, 32). Detailed analysis shows, however, that not the numerical age as such but the number and nature of the underlying disorders determine the outcome (33). Among the disorders involving a higher postoperative risk are, above all, disturbances of cardiac, pulmonary, renal and hepatic functions, diabetes mellitus, obesity and malignant disease (34).

In our prospective study on patients suffering from secondary peritonitis the distribution of gender and age corresponded well to data given in another clinical trial (2). Both in our own total test group and in the study described by *Billing et al.* (2), but in contradiction to data of *Lindner et al.* (5), female patients had a tendency (87 %) towards better survival compared to males (73 %). A sex specific incidence and outcome of septic multiple organ failure was also claimed by *Oberholzer et al.* (35) just recently. Moreover, patient age is accepted as a relevant factor for prediction of outcome in some scores (5, 6, 7, 36, 37). Yet, in our patient subgroups of survivors and non-survivors no significant age-differences became obvious. The concurrent prevalence of an underlying malignant disorder has also been repeatedly described as an unfavorable factor (2, 33). However, the proportion of malignant tumors was slightly lower (27 %) in our group of non-survivors than in the group of survivors (36 %).

*Clinical Scores.* The *MPI* is the only clinical score system considering the intraperitoneal evaluation. Similar to the other scores which can be calculated throughout the whole observation period, mortality increases in line with the rising number of score points of the MPI (5, 38, 39). Yet in our study, no predictive value could be attributed to the MPI showing likewise high points in both outcome groups. This confirms the common clinical experience that the MPI is well suited for the local characterization of peritonitis but unlike to APACHE II, MOF and SOFA scores, plays a subordinate role as an initial prediction parameter.

Various studies on peritonitis have demonstrated that the APACHE II score correlates with postoperative morbidity and mortality and, in addition reflects the de-

gree of severity with great accuracy (2, 37, 39–46). The evaluation of the different score systems in our own investigation revealed a most accurate prediction of peritonitis-related death by high points of *SOFA* followed by *MOF* and *APACHE II* score, both for the preoperative value and the postoperative 4 days. However, in agreement with other authors, we found that none of the score systems had at the same time sufficient sensitivity *and* specificity to be used exclusively as a prognostic instrument (2, 6, 43, 47). Nevertheless, in the absence of any better option, it seems reasonable to use scores for clinical decision-making and for comparing multicenter studies (30, 43).

*Hemostasis.* Both significantly longer *partial thromboplastin time* (PTT) and a highly significant drop in *thrombocyte count* in the group of the deceasing patients confirms the predictive relevance of these factors as described already by other investigators (40, 48, 49). In contrast, coagulation parameters such as prothrombin time (*Quick*) and latent thrombin activity (*prothrombin*) showed only a minor predictive usefulness in certain time segments during the observation phase.

In cases of lethal sepsis due to MOF, the systemic thrombomodulin concentration in the plasma increased substantially as a consequence of the massive damage to the endothelial cells (10, 50). This observation was verified by our patient collective eventually dying from secondary peritonitis. Even at the preoperative stage prior to the primary operation, the *thrombomodulin* values were already significantly elevated in the group of non-survivors.

*Leukocyte Count, Proteinases, Acute Phase Proteins, Cytokines, and Adhesion Molecules.* The release of the destructive proteinases *PMN-elastase* from neutrophil granulocytes and *cathepsin B* from monocytes/macrophages correlates closely to the inflammatory response (11, 12, 28, 51). Quantification of these proteinases as indicators of systemic inflammation revealed a substantially yet not statistically significantly higher plasma level in non-survivors of our study in accordance to preliminary data shown by *Holzheimer et al.* (52) for peritonitis patients. Moreover, *procalcitonin* levels of our patients differed with clearly higher significance between lethal and reversible organ failure, both at the preoperative stage and during the 1st time segment of observation. Although cellular origin and pathogenetic significance of procalcitonin are largely unknown, this protein seems to be one of the main inflammatory parameter with prognostic significance in bacterial inflammation (13, 53-55). Interestingly, in our patient collective neither leukocyte count nor CRP provided a meaningful prognosis of the patients' outcome although both parameters were highly elevated during the early observation phase. In contrast, *Goetz et al.* (56) demonstrated a clear correlation between CRP and the severity of the peritonitis, which might be due to a more pronounced general bacterial sepsis in their patients.

The pro- (IL-6, IL-8) or anti-inflammatory (IL-1ra, TNF-RII) parameters of the cytokine system remained at a higher level in the non-survivors during the early

study period in accordance with results published by *van Deuren et al.* (57) on meningococcal infections. Yet, in our peritonitis patients only IL-8 and TNF-RII differentiated highly significantly between both outcome groups thus corroborating data published by *Ertel et al.* (22) in septic patients. Other authors, however, have also demonstrated a significant correlation of IL-6 plasma concentrations with sepsis-associated mortality and with the degree of organ dysfunction in septic patients (38, 45, 46, 52, 58-64). Although not statistically significant, the decrease in the *IL-6* concentration in the group of survivors was associated at least with clinical improvement and a lower incidence of complications compared to non-survivors in our study.

Concerning the concentration of the soluble *ICAM-1* and selectins, we expected a clear correlation of these adhesion molecules with the severity of the peritonitis according to data provided by *Gearing and Newmann* (21), *Pruimboom et al.* (65), *Cowley et al.* (66) and *Inthorn et al.* (17) in septic subjects. Yet, in our patients no significant preoperative or postoperative differences between survivors and non-survivors could be found for ICAM-1 or for any of the other adhesion molecules. Interestingly, however, soluble ICAM-1 and *L-selectin* showed lower concentrations in the group of non-survivors, which may indicate an enhanced binding of the soluble molecules to activated endothelial or other inflammation cells (67).

*Parameters of Opsonization.* Even at the preoperative stage, a significant difference existed between survivors and non-survivors concerning complement C3 as an essential opsonization parameter, remaining at an extremely low, pathological level throughout the entire observation period in the patients who eventually died. Similar results were obtained for the *opsonization capacity*. In contrast, no differences between the groups became obvious for IgG and fibronectin. These results are in line with those published by *Billing et al.* (23) suggesting that C3 *in vivo* may play a much more important role in the opsonization and defense against bacteria than the latter two opsonins.

*Organ Function Parameters.* Most of the indicators of specific organ functions displayed a high significance for the prediction of outcome already at the preoperative stage. Thus, the preoperative levels of *creatinine* and *neopterin* measuring renal function showed pathological increases primarily in non-survivors. These data coincide well with those published by other authors, too (18, 26, 75, 68, 69). Moreover, the $pO_2/FiO_2$ *ratio* as a criteria of the pulmonary capacity, differentiated significantly between the groups as early as at the patients admission, thus confirming data of *Vincent et al.* and other authors (7, 8, 70). *P-III-P* providing information on the current fibrotic transformation of liver tissue and/or the eliminatory function of the liver (24), also demonstrated significantly higher initial values in the group of non-survivors at the time of the primary operation and throughout the following 4 day observation period. Similar data concerning early prognosis of outcome in severely injured patients have been published by *Waydhas et al.* (25). In contrast, serum levels of bilirubin, the common indicator of the

hepatic clearance, revealed poor prognosis only when the whole 4 day observation period was considered for statistical evaluation despite clearly higher levels in non-survivors at any time.

*Combination of Clinical Score Systems and Significant Laboratory Parameters.* Unlike data of other study groups which favor individual prediction factors such as phospholipid A or PMN-elastase in peritonitis patients (71) respectively procalcitonin in septic patients (16), our study demonstrated that a wide variety of clinical scores and inflammation parameters display a reasonable usefulness for prediction of outcome in the special clinical setting of peritonitis. After evaluating the common score systems comprising several clinical and routine laboratory parameters with logistic regression analysis, we were able to show that the SOFA is superior in predicting outcome of peritonitis. These findings corroborate those of other authors (9, 72-74), although the SOFA score was originally not designed to evaluate risk of death (8). In contrast to data presented by *Cerra et al.* (70) and *Barie et al.* (41) also the APACHE II predicted mortality in our patient collective with reasonable certainty. In addition, we could demonstrate for the first time using the logistic regression analysis that prediction of outcome can be increased further by the combination of preoperative SOFA score and various inflammation parameters. The combination of preoperative SOFA score and neopterin level yielded the highest sensitivity (64 %) for predicting lethal outcome (specificity: 93 %; PPV: 70 %; NPV: 91 %). Neopterin is a metabolic product of macrophages and represents their degree of activity provided that an intact kidney function exists. Yet, if the renal elimination is disturbed due to an inflammatory hit, a high neopterin level must be mainly explained as an early sign of kidney failure (27). Thus, the correlation of increased neopterin levels with the occurrence and severity of a posttraumatic or postoperative septic renal failure has also been described in several studies (18, 26, 69, 75).

If the mean values of the first 4 postoperative days are calculated for each patient, the combination of TNF-RII and SOFA-Score had the highest sensitivity (73 %) for the lethal outcome of secondary peritonitis (specificity: 96 %; PPV: 80 %; NPV: 93 %). This points towards the pathogenic significance of a permanent inflammatory signal transmission via the TNF-RII (22, 58, 76, 77).

Summarizing the results of our study and those of other authors it becomes obvious that the patients with abdominal sepsis represent a major portion of long-term ICU patients. With advancing improvements in intensive care, multiple organ failure (MOF) gains increasing importance for the death of patients with abdominal sepsis. It is then no longer the septic shock, but the protracted sepsis syndrome known as tertiary peritonitis. Regarding the predictive relevance of clinical scores and numerous biochemical inflammation parameters, the data obtained allow the following conclusions with respect to lethal outcome. Parameters for an unfavorable outcome include drop in thrombocyte count and longer partial thromboplastin time (PTT), increase of the systemic thrombomodulin level, persistent hyperreactive cytokine response (pro- or anti-inflammatory), and increased binding of

soluble L-selectin and ICAM-1 to activated endothelial cells with a consecutive decrease in serum levels. Yet, optimal prediction is not provided by a single risk factor, but by a combination of SOFA score and neopterin preoperatively or of SOFA score and TNF-RII at the early postoperative stage (days 0 to 4) respectively.

However, with respect to routine practicability of our suggested scoring system, further clinical studies in larger patient populations with secondary peritonitis are indicated employing single morning sampling instead of 2 to 6 hourly sampling performed in this first evaluation study.

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**Table 1.** Preoperative characteristics of peritonitis patients stratified into non-survivors and survivors.

| Parameter | Non-survivors (n=11) | Survivors (n=45) | p-Value |
|---|---|---|---|
| Age (Years) * | 65.6 (± 4.9) | 58.2 (± 2.1) | 0.2 |
| Gender (f/m) # | 4/7 | 26/19 | 0.4 |
| Malignant tumor (yes/no) # | 3/8 | 16/29 | 0.8 |

* Data are given as mean value (± SEM). Wilcoxon-U-test or # Chi-Quadrat-test were used for statistics.

**Table 2.** Origin of the peritonitis.

| Organ of origin | Total (n=56) | Non-survivors (n=11) | Survivors (n=45) |
|---|---|---|---|
| Colon and rectum | 31 (55) | 6 (55) | 25 (56) |
| Jejunum/ileum | 14 (25) | 3 (27) | 11 (24) |
| Stomach/duodenum | 9 (16) | 1 (9) | 8 (18) |
| Others | 2 (4) | 1 (9) | 1 (2) |

Data are given as numbers of affected patients and distribution (%) within the groups.

**Table 3.** Germ spectrum during primary intervention.

| **Germs** | **Total** (n=56) | **Non-survivors** (n=11) | **Survivors** (n=45) |
|---|---|---|---|
| Aerobic | 16 (28.6) | 2 (18.2) | 14 (31.1) |
| Aerobic + anaerobic | 28 (50.0) | 7 (63.6) | 21 (46.7) |
| Candida albicans | 6 (10.7) | 2 (18.2) | 4 (8.9) |
| None | 6 (10.7) | 0 | 6 (13.3) |
| E. coli | | | |
| without bacteroids | 32 (57.1) | 4 (36.4) | 28 (62.2) |
| with bacteroids | 24 (42.9) | 7 (63.6) | 17 (37.8) |

Data are given as numbers of patients and distribution (%) within the groups.

**Table 4.** Intra-operative value of MPI and preoperative values of APACHE II, MOF and SOFA score of peritonitis patients.

| **Score** | **Non-survivors** (n=11) | **Survivors** (n=45) | **p-value** |
|---|---|---|---|
| MPI | 29.3 (2.3) | 27.2 (0.9) | 0.235 |
| **APACHE II** | **18.4 (1.6)** | **12.5 (0.6)** | **0.003** |
| **MOF** | **5.7 (0.7)** | **2.8 (0.4)** | **0.001** |
| **SOFA** | **7.5 (1.1)** | **3.2 (0.4)** | **0.0005** |

Data are given as mean value (± SEM): Bold type indicates significant group differences (Wilcoxon-U-test).

**Table 5.** Comparison of APACHE II, MOF and SOFA scores for time segment I (day 0-4) of peritonitis patients.

| Score | Non-survivors (n=11) | Survivors (n=45) | p-value |
|---|---|---|---|
| **APACHE II** | **17.4 (1.6)** | **11.2 (0.6)** | **0.014** |
| **MOF** | **6.3 (0.6)** | **3.0 (0.3)** | **0.001** |
| **SOFA** | **10.6 (1.0)** | **5.3 (0.4)** | **0.0001** |

Data are given as mean value (± SEM): Bold type indicates significant group differences (Wilcoxon-U-test).

**Table 6.** Preoperative values of inflammation parameters in the circulation of peritonitis patients.

| *Parameters* | Non-survivors (n=11) | Survivors (n=45) | p-Value |
|---|---|---|---|
| *Coagulation* | | | |
| **Thrombocytes/nl** | **243.0 (39.0)** | **349.0 (21.0)** | **0.04** |
| **PTT (sec.)** | **51.9 (7.9)** | **37.9 (1.5)** | **0.05** |
| Quick (%) | 65.5 (7.1) | 71.4 (3.3) | 0.324 |
| **Thrombomodulin (ng/ml)** | **127.0 (19.2)** | **62.9 (5.3)** | **0.0005** |
| prothrombin ( %NP) | 57.7 (4.9) | 65.8 (2.8) | 0.146 |

| | | | | | |
|---|---|---|---|---|---|
| *Leukocyte Count, Proteinases, Acute Phase Reactions* | | | | | |
| Leukocytes x1000/µl | 12.8 | (2.9) | 16.5 | (1.7) | 0.238 |
| Elastase (ng/ml) | 583.6 | (139.8) | 699.7 | (70.1) | 0.312 |
| Cathepsin B (mU/l) | 123.9 | (18.2) | 128.5 | (23.5) | 0.287 |
| CRP (mg/dl) | 10.5 | (1.9) | 11.6 | (1.0) | 0.675 |
| **Procalcitonin (ng/ml)** | **50.8** | **(21.7)** | **22.7** | **(9.4)** | **0.013** |
| *Cytokine System* | | | | | |
| IL-1ra (pg/ml) | 39412 | (11714) | 41620 | (13793) | 0.255 |
| IL-6 (pg/ml) | 3377 | (1299) | 2192 | (1036) | 0.154 |
| **IL-8 (pg/ml)** | **1444** | **(514)** | **445** | **(194)** | **0.007** |
| **TNF-RII (ng/ml)** | **32.8** | **(6.5)** | **17.3** | **(1.4)** | **0.007** |
| *Opsonization* | | | | | |
| IgG (mg/dl) | 518.5 | (59.5) | 558.7 | (35.1) | 0.621 |
| **C3 (mg/dl)** | **34.9** | **(2.9)** | **55.2** | **(11.5)** | **0.04** |
| **Opsonization capacity (%)** | **59.1** | **(12.0)** | **88.9** | **(6.2)** | **0.026** |
| Fibronectin (mg/dl) | 12.3 | (2.0) | 14.3 | (0.9) | 0.417 |
| *Organ function* | | | | | |
| **Creatinine (mg/dl)** | **2.4** | **(0.4)** | **1.1** | **(0.1)** | **0.0002** |
| **Neopterin (nmol/l)** | **128.5** | **(33.5)** | **33.3** | **(5.7)** | **0.0001** |
| **pO2/FiO2** | **154.6** | **(32.6)** | **299.0** | **(21.9)** | **0.003** |
| Bilirubin (mg/dl) | 2.0 | (0.9) | 1.6 | (0.3) | 0.753 |
| **P-III-P (E/ml)** | **3.9** | **(0.8)** | **2.2** | **(0.5)** | **0.003** |

Data are given as mean value (± SEM): Bold type indicates significant group differences (Wilcoxon-U-test). PTT, partial thromboplastin time; NP, normal plasma; CRP, C-reactive protein; IL-1ra, interleukin-1 receptor antagonist; TNF-RII, tumor necrosis factor receptor II; IgG, immunoglobulin G; C3, complement C3; P-III-P, procollagen-III-peptide.

**Table 7.** Parameters of inflammation in the circulation of peritonitis patients during time segment I (day 0-4).

| *Parameters* | Non-survivors (n=11) | | Survivors (n=45) | | p-Value |
|---|---|---|---|---|---|
| *Coagulation* | | | | | |
| **Thrombocytes /nl** | **168.0** | **(27.0)** | **274.0** | **(17.0)** | **0.009** |
| **PTT (sec.)** | **51.5** | **(3.7)** | **41.2** | **(0.8)** | **0.001** |
| **Quick (%)** | **58.2** | **(5.4)** | **69.5** | **(1.9)** | **0.005** |
| **Thrombomodulin (ng/ml)** | **137.0** | **(17.9)** | **77.8** | **(7.1)** | **0.001** |
| prothrombin (% NP) | 60.4 | (2.6) | 65.4 | (1.8) | 0.183 |
| *Leukocyte Count, Proteinases, Acute Phase Reactions* | | | | | |
| Leukocytes x1000/µl | 16.3 | (2.3) | 15.1 | (1.0) | 0.516 |
| Elastase (ng/ml) | 577.3 | (87.1) | 408.1 | (28.6) | 0.269 |
| **Cathepsin B (mU/l)** | **163.5** | **(39.6)** | **77.6** | **(5.8)** | **0.038** |
| CRP (mg/dl) | 12.6 | (1.8) | 12.2 | (0.6) | 0.845 |
| **Procalcitonin (ng/ml)** | **76.2** | **(44.9)** | **19.6** | **(9.1)** | **0.004** |
| *Cytokine System* | | | | | |
| IL-1ra (pg/ml) | 25756 | (6067) | 15108 | (2445) | 0.146 |
| IL-6 (pg/ml) | 2917 | (1218) | 710 | (290) | 0.072 |
| **IL-8 (pg/ml)** | **1527** | **(499)** | **169** | **(61)** | **0.0001** |
| **TNF-RII (ng/ml)** | **33.1** | **(4.3)** | **16.5** | **(1.4)** | **0.0004** |
| *Opsonization* | | | | | |
| IgG (mg/dl) | 646.3 | (61.6) | 574.4 | (29.0) | 0.278 |
| **C3 (mg/dl)** | **32.0** | **(2.1)** | **46.8** | **(8.3)** | **0.05** |
| Opsonization capacity (%) | 70.6 | (8.9) | 83.4 | (5.2) | 0.223 |
| Fibronectin (mg/dl) | 13.4 | (1.3) | 13.0 | (0.6) | 0.375 |
| *Organ function* | | | | | |
| **Creatinine (mg/dl)** | **2.4** | **(0.4)** | **1.1** | **(0.1)** | **0.0001** |
| **Neopterin (nmol/l)** | **134.3** | **(20.6)** | **43.1** | **(7.9)** | **0.0001** |
| **pO2/FiO2** | **190.2** | **(27.9)** | **282.5** | **(13.9)** | **0.01** |
| **Bilirubin (mg/dl)** | **2.9** | **(0.6)** | **1.7** | **(0.2)** | **0.03** |
| **P-III-P (E/ml)** | **5.0** | **(1.3)** | **2.9** | **(0.6)** | **0.006** |

Data are given as mean value (± SEM): Bold type indicates significant group differences (Wilcoxon-U-test). PTT, partial thromboplastin time; NP, normal plasma; CRP, C-reactive protein; IL-1ra, interleukin-1 receptor antagonist; TNF-RII, tumor necrosis factor receptor II; IgG, immunoglobulin G; C3, complement C3; P-III-P, procollagen-III-peptide.

**Table 8.** Calculation of the survival probability of peritonitis patients using the preoperative values of neopterin (nmol/l) and the SOFA score.

| SOFA score | (0–6) | (7–12) | (13–18) | (19–24) |
|---|---|---|---|---|
| Neopterin | | | | |
| (0–100) | **0.9329** | 0.5852 | 0.1252 | 0.0143 |
| (101–200) | 0.6471 | **0.1569** | 0.0185 | 0.0019 |
| (201–300) | 0.1948 | 0.0239 | **0.0025** | 0.0003 |
| (301–400) | 0.0309 | 0.0032 | 0.0003 | **0.00003** |

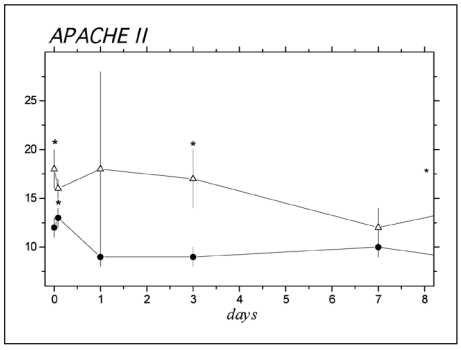
For calculation the following equation was used: $R = 1/(1 + e^{-4.81 + 0.02 \times [\text{neopterin}] + 0.44 \times \text{SOFA score}})$.

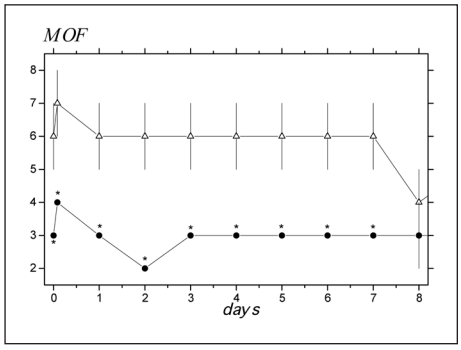
**Table 9.** Calculation of the survival probability of peritonitis patients using the mean values of TNF-RII (ng/ml) and the SOFA score during the time segment I (day 0-4).

| SOFA score | (0–6) | (7–12) | (13–18) | (19–24) |
|---|---|---|---|---|
| TNF-RII | | | | |
| (0–15) | **0.9696** | 0.7581 | 0.2355 | 0.0294 |
| (16–30) | 0.9683 | **0.7503** | 0.228 | 0.0282 |
| (31–45) | 0.967 | 0.7425 | **0.2208** | 0.0271 |
| (46–60) | 0.9656 | 0.7344 | 0.2137 | **0.026** |

For calculation the following equation was used: $RI = 1/(1 + e^{-6.49 + 0.06 \times [\text{TNF RII}] + 0.64 \times \text{SOFA score}})$.

TNF-RII, tumor necrosis factor receptor II.



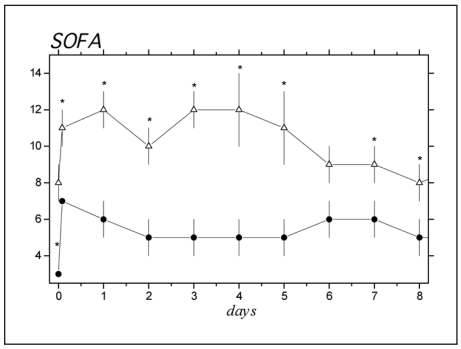




*Figure 1:*

*APACHE II, MOF and SOFA score in peritonitis patients.*

*Follow-up values for non-survivors (D, n = 11) and survivors (l , n = 45) are given as mean value (± SEM). * Wilcoxon-U-test, p < 0.05*

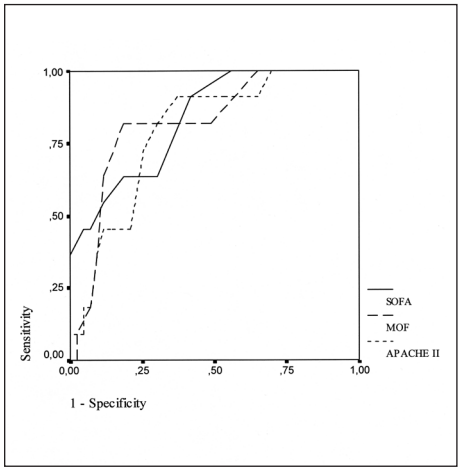



*Figure 2:*

*ROC-curves for the preoperative values of different clinical scores measured in peritonitis patients. Areas under the curve:*
*APACHE II = 0.79; MOF = 0.82; SOFA = 0.83*

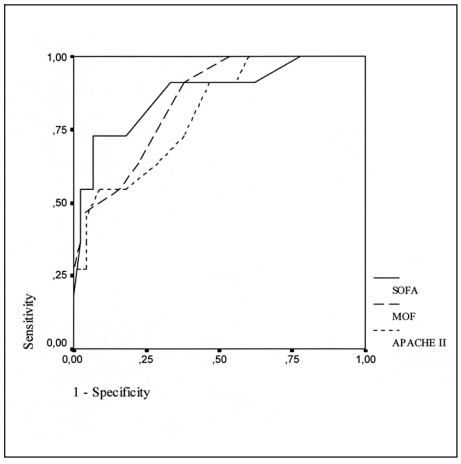
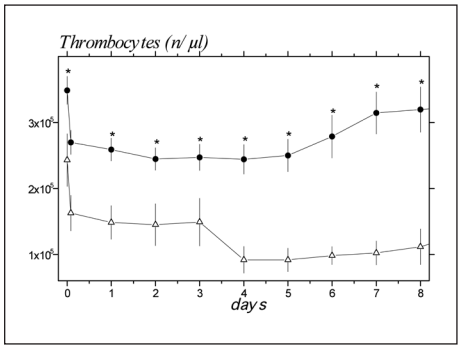



*Figure 3:*

*ROC-curves for the time segment I (day 0-4) of different clinical scores measured in peritonitis patients.*

*Areas under the curve:*
*APACHE II = 0.81; MOF = 0.85; SOFA = 0.87*



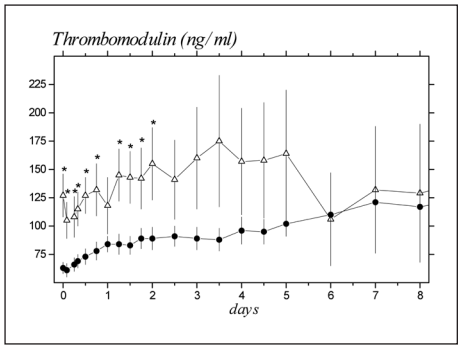




*Figure 4*

*Coagulation parameters in the circulation of peritonitis patients.*

*Follow-up values for non-survivors (Δ, n = 11) and survivors (• , n = 45) are given as mean value (± SEM). * Wilcoxon-U-test, p < 0.05*

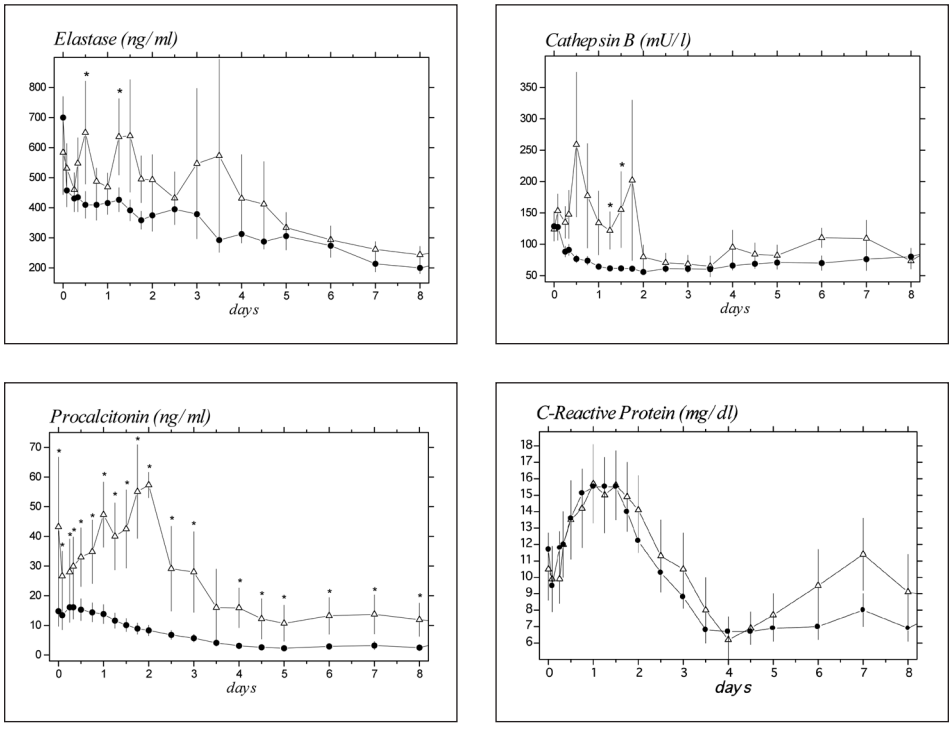



*Figure 5:*

*Leukocyte proteinases and acute phase proteins in the circulation of peritonitis patients.*

*Follow-up values for non-survivors (Δ, n = 11) and survivors (• , n = 45) are given as mean value (± SEM). * Wilcoxon-U-test, p < 0.05*

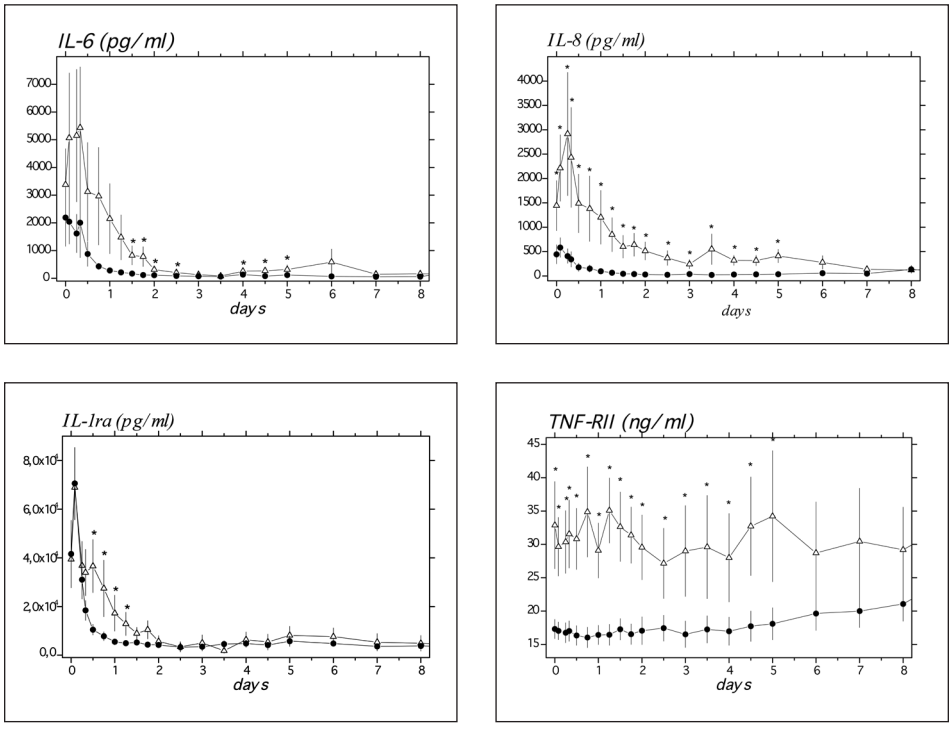



*Figure 6:*

*Parameters of the cytokine system in the circulation of peritonitis patients. Follow-up values for non-survivors (Δ, n = 11) and survivors (• , n = 45) are given as mean value (± SEM). * Wilcoxon-U-test, p < 0.05*

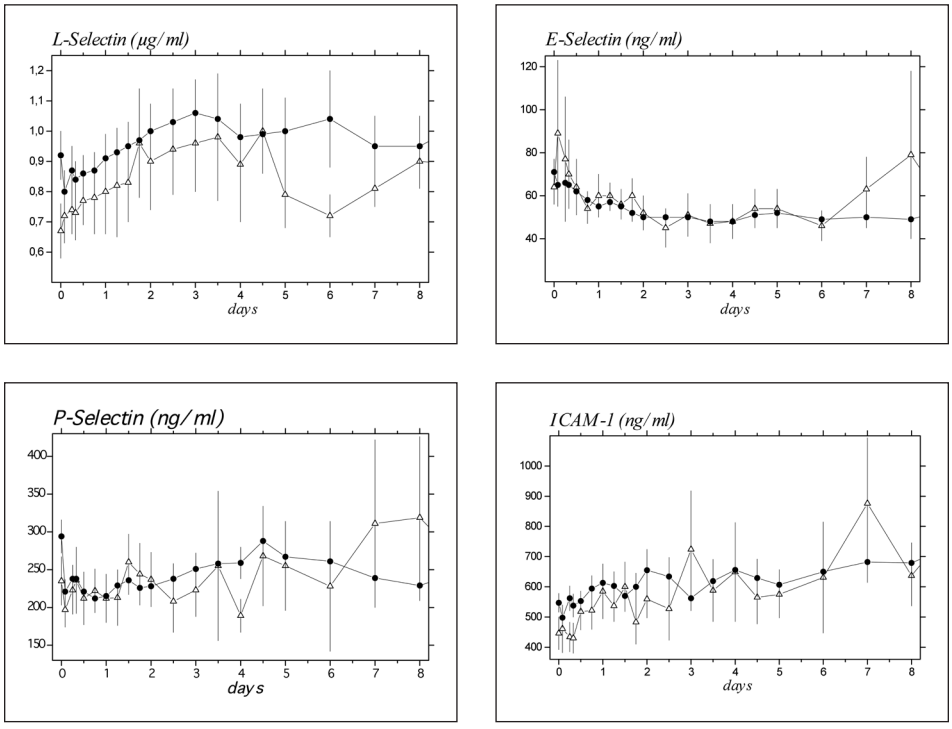



*Figure 7:*

*Soluble adhesion molecules in the circulation of peritonitis patients.*

*Follow-up values for non-survivors (Δ, n = 11) and survivors (• , n = 45) are given as mean value (± SEM). * Wilcoxon-U-test, p < 0.05*

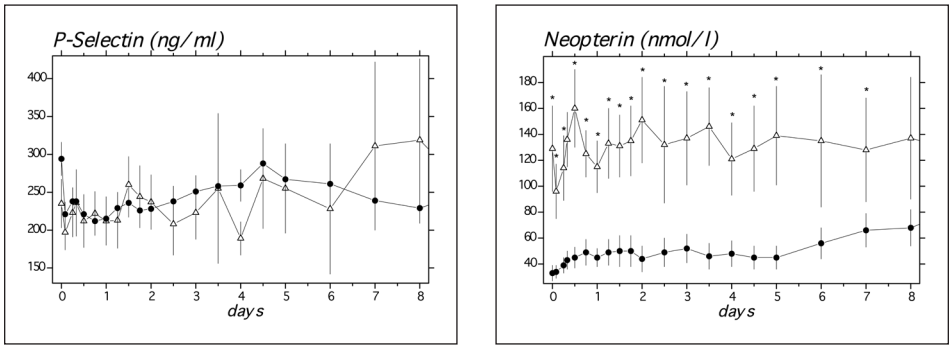



*Figure 8:*

*Organ function parameters in the circulation of peritonitis patients.*

*Follow-up values for non-survivors (Δ, n = 11) and survivors (• , n = 45) a are given as mean value (± SEM). * Wilcoxon-U-test, p < 0.05*

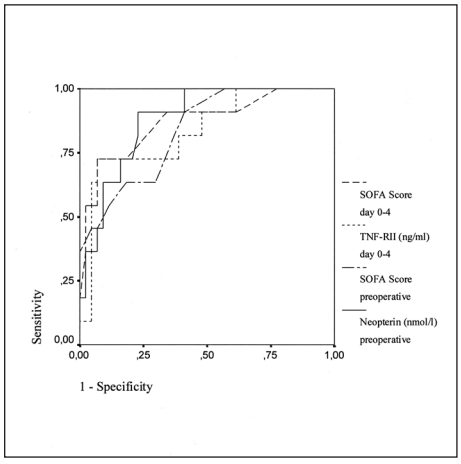



*Figure 9:*

*ROC-curves for the preoperative values and the time segment I (day 0-4) of inflammation parameters with the highest outcome prediction in peritonitis patients. Areas under the curve: preoperative: Neopterin (0.88) and SOFA (0.87); time segment I: TNF-RII (0.84) and SOFA (0.84)*