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Article

An evaluation of plasma paraoxonase-1 (PON-1) levels in young and middle-aged patients with type-2 diabetes mellitus

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Abstract: Objectives: There is a lot of evidence that oxidative stress plays a role in the etiology of aging. PON-1 is mostly complexed to HDL and is responsible for its antioxidant properties. This investigation was undertaken to assess age-dependent changes in plasma PON-1 concentration and its association with age, HDL, BMI, and duration of diabetes among T2DM patients.
Methods: This study was carried out on 125 clinically diagnosed T2DM patients (young and middle-aged) attending the OPD and IPD of Rohilkhand Medical College and Hospital. Laboratory investigations such as fasting plasma glucose (FPG), HbA1c, and plasma PON-1 were measured.
Results: When compared with young patients, middle-aged patients showed a significantly declined plasma PON-1 ($p < 0.01$) and HDL ($p < 0.05$) levels and increased BMI ($p < 0.05$). The young and middle-aged patients showed a negative correlation of PON-1 with BMI ($p < 0.01$), duration of diabetes ($p < 0.01$), and a positive correlation with HDL ($p < 0.01$). However, only middle-aged diabetic patients showed a significant correlation of PON-1 with FPG ($p < 0.01$) and HbA1c ($p < 0.01$).
Conclusion: Decreased PON-1 concentration in middle-aged patients might be due to a decrease in HDL levels as a consequence of oxidative stress since PON-1 is mainly complexed to HDL.

Keywords: Plasma PON-1; Ageing; Diabetes mellitus type-2; Oxidative stress.

1. Introduction

Several hypotheses have arisen throughout the years to better comprehend the process of ageing. Among these, the effect of oxidative stress on ageing has garnered a lot of attention. It is based on the fact that cell metabolism generates harmful bioproducts, free radicals, and oxidising metabolites even under physiological circumstances. The commencement of the ageing process would be caused by an imbalance between the synthesis of oxidative metabolites and the presence of antioxidants. Free radicals, Reactive Oxygen Species (ROS) and/or Reactive Nitrogen Species (RNS), and peroxides cause oxidative damage to macromolecules (carbohydrates, lipids, proteins, and DNA) in both the intracellular and extracellular microenvironments [1].

The elderly are more vulnerable to oxidative damage due to a decrease in molecules that protect against oxidation [2]. Several nutrients, such as antioxidant vitamins (vitamin A and -carotene, vitamin C and E) and polyphenolic compounds, are more difficult for older people to absorb or synthesise [3]. Additionally, antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and paraoxonases (PONs) exhibit decreased activity with ageing [4].

Being an oxidative stress state, diabetes mellitus makes use of antioxidant resources of the body. One such antioxidant is the PON-1 enzyme, which is related to high density lipoprotein (HDL). PON-1 serum activity is significantly decreased in patients with a number of diseases, including ischemia, chronic kidney disease,

morbid obesity, and dyslipidemias [5], but only a small number of studies have found age-related changes in PON-1 concentration, particularly in relation to diabetes.

Thus, as PON-1 is an antioxidant enzyme we predicted that PON-1 concentration could decline with ageing. Therefore, the aim of the study was to assess the effect of age on levels of PON-1 in patients with type-2 diabetes mellitus (T2DM). Moreover, to investigate factors that might alter PON-1 concentration with ageing and finally the correlation of PON-1 with ageing, HDL, BMI and duration of disease.

2. Material and methods

2.1. Patient selection

A total of 125 samples from T2DM patients were collected from outpatient department at Rohilkhand Medical College and Hospital in Bareilly. Patients were subcategorized into young (30-45 years) and middle aged (46-60 years) groups. All participants provided their informed consent, and the research was endorsed by the Institutional Ethics Committee. Study participants with chronic kidney diseases, hepatic diseases, cerebrovascular disorders, cardiopulmonary arrest, gestational diabetes, chronic inflammation, infection, hypertension and tuberculosis were excluded from the study.

2.2. Biochemical investigations

In accordance with the standards and guidelines established by the American Diabetes Association (ADA), T2DM was formally diagnosed [6]. About 5ml of venous blood samples were drawn under aseptic precautions after overnight or 8 hours fasting from all subjects and dispersed into fluoride vial for plasma glucose estimation and EDTA (ethylene diamine tetra-acetic acid) vial for HbA1c and plasma PON-1 estimation. The glucose oxidase and peroxidase (GOD-POD) method [7] was employed to determine fasting plasma glucose by Erba semi auto analyzer and HbA1c estimation was done by Biorad D10 analyzer utilizing ion-exchange high performance liquid chromatography (HPLC) technique [8].

2.3. Human plasma PON-1

A sandwich enzyme linked immuno-sorbent assay (ELISA) kit from Elabscience was employed to estimate the plasma PON-1 concentration in a manner conforming to manufacturer's instructions. Plasma PON-1 levels were measured and the remaining sample were stored at -20°C for further analysis. 100 μL of standards and samples were taken in appropriate wells of PON-1 pre coated ELISA microtiter plate and incubated at 37°C for 90 minutes. Then, samples and standards were removed and 100 μL of Biotinylated detection antibody specific for PON-1 were added and incubated at 37°C for one hour. After that wells were washed 3 times by using wash buffer and 100 μL of Horse Reddish Peroxidase (HRP) conjugate secondary antibody was added and incubated at 37°C for half an hour. Wells were washed by 5 times again followed by the addition of 90 μL substrate reagent and incubated at 37°C for 15 minutes. Only the wells containing PON1 biotinylated detection antibody and avidin-HRP conjugate turned blue. 50 μL stop solution was added in each well to cease the enzyme substrate reaction that changes color to yellow and optical density (OD) were measured immediately at a wavelength of 450nm using ELISA reader. PON-1 concentration in samples was determined by putting the ODs on a standard curve.

2.4. Statistical analysis

Data was collected and compiled using Microsoft Excel. The statistical package for social services (SPSS) software program, 29.0 version, was used to carry out all statistical analysis. The date was normally distributed and presented as mean ± SD for all parameters. The mean between young and middle-aged patients with T2DM was compared using the Student's t-test. The relationship between the research variables was established using Pearson correlation. Statistical significance was considered as a probability value (p) less than 0.05.

3. Results

Patients with T2DM were subcategorized into two age groups; young (30-45 years) and middle-aged (46-60 years). The demographic and laboratory investigation of studied groups according to age were summarized in Table 1. The mean age of young and middle-aged patients was 39.68 ± 5.92 and 53.88 ± 5.66 years respectively.

The middle-aged patients showed significantly lower plasma PON-1 ($p < 0.01$) and HDL ($p < 0.05$) levels in comparison to young patients with T2DM. BMI was found to be significantly higher ($p < 0.05$) in middle aged diabetic patients when compared to young diabetic patients.

The young and middle-aged patients showed negative correlation of PON-1 with BMI ($p < 0.01$), duration of diabetes ($p < 0.01$), and positive correlation with HDL ($p < 0.01$). However, only middle-aged diabetic patients showed significant correlation of PON-1 with FPG ($p < 0.01$) and HbA1c ($p < 0.01$).

Table 1. Demographic and laboratory investigations of studied groups

Parameters	Patients with T2DM (n = 125)		
	Young (30-45 years) (n = 44)	Middle-aged (46-60 years) (n = 81)	p –value
Age (years)	39.68 ± 5.92	53.88 ± 5.66	0.000**
BMI (kg/m2)	26.44 ± 4.43	28.15 ± 3.24	0.015*
Weight (kg)	68.89 ± 8.03	72.48 ± 7.87	0.017*
Duration of T2DM (years)	3.52 ± 2.07	5.16 ± 2.3	0.000**
FPG (mg/dl)	213.45 ± 81.15	224.35 ± 72.64	0.444
HbA1c (%)	8.89 ± 2.36	9.49 ± 2.42	0.186
PON-1 (ng/ml)	2.74 ± 2.21	1.55 ± 1.45	0.000**
HDL (mg/dl)	34.79 ± 9.62	29.95 ± 8.13	0.003*

*. Significant at the level 0.05 (2-tailed) **. Significant at the level 0.01 (2-tailed)

Table 2. Correlation of PON-1 with age, BMI, HDL, disease duration, FPG and HbA1c among T2DM patients of age group 30-45 years and 46-60 years

Parameters	Young (30-45 years)		Middle-aged (46-60 years)	
	r-value	p-value	r-value	p-value
PON-1 and Age	-0.210	0.172	0.192	0.085
PON-1 and BMI	-0.507	0.000**	-0.332	0.002**
PON-1 and HDL	0.845	0.000**	0.873	0.000**
PON-1 and duration of diabetes	-0.609	0.000**	-0.497	0.000**
PON-1 and FPG	-0.275	0.071	-0.427	0.000**
PON-1 and HbA1c	-0.231	0.131	-0.368	0.001**

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

An area of interest is the existence and significance of elevated extracellular and intracellular oxidative stress during ageing and diseases associated with old age [9,10]. Clinical studies have confirmed and validated the antioxidant functions of PON-1 [11]. The aetiology of several diseases, including malignancies, renal diseases, Parkinson's disease, Alzheimer's disease, liver disease, and diabetes mellitus, has been associated with oxidative imbalance [12].

Present work demonstrate the significant reduction ($p < 0.01$) of plasma PON-1 (Figure 1) concentration in middle-aged (46-60 years) patients with T2DM when compared with young age (30-45 years) patients. Similar findings were also given by various other studies [13–15] indicating significant age related changes. However, Seres et al. [14] demonstrated a decrease in PON-1 activity without a change in its serum concentration and this decrease was linked to development of oxidative stress with ageing.

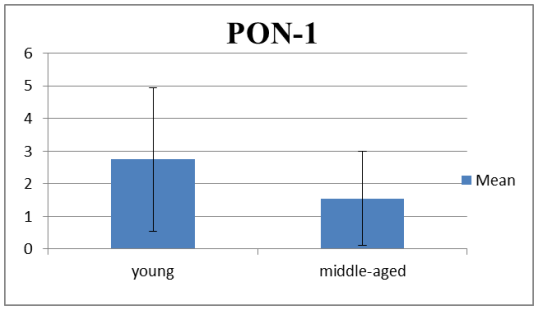

Figure 1. Comparison of PON-1 in young and middle aged patients with T2DM

Previous studies have shown that reduced PON-1 activity and serum concentration are related to establish T2DM [16,17]. PON-1 specific activity has been reported to be reduced by non-enzymatic glycation [18]. However, relatively few studies have examined how the PON-1 concentration varies with ageing in diabetes, particularly in T2DM [19]. The current study cannot give a definitive explanation for the decrease in PON-1 concentration in middle-aged patients. However, it may be suggested that the decline in PON-1 enzyme concentration with ageing is due to an increase in oxidative stress. It is possible that genotype influences the activity and concentration of PON-1 in human serum; interspecies variations may account for the various PON-1-related ageing mechanisms.

The development and progression of diabetes and its complications have both been linked to oxidative stress. Long-term oxidative damage has three main effects: mitochondrial malfunction, β-cell dysfunction, and impaired glucose tolerance. The decrease in serum PON-1 activity produced by oxidative stress has been linked mostly to changes in the redox status of the protein's free sulfhydryl group, which prevents reactive oxygen species from inhibiting PON-1 function [20].

In order to further explain reduction in PON-1 concentration with age we also estimated HDL levels (Figure 2) as a function of age. Indeed, HDL is the principle PON-1 carrier in plasma. Our results shows a significant reduction in HDL concentration in middle-aged ($p < 0.05$) patients when compared with young individuals. In plasma, PON-1 is entirely complexed to HDL thus the reduction in PON-1 concentration could be due to a reduction in HDL concentration with aging in patients with T2DM. Previous research has shown that HDL oxidation susceptibility increases with ageing [21]. T2DM is characterised by low levels of HDL-cholesterol, according to Martinez Castelao et al. [22].

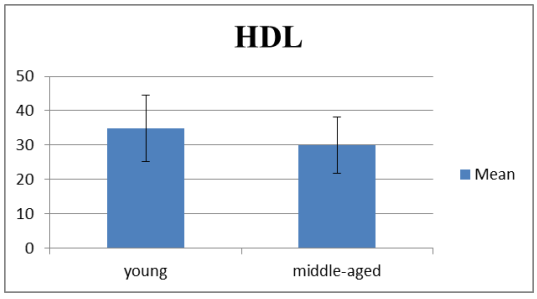

Figure 2. Comparison of HDL in young and middle aged patients with T2DM

Reduced plasma concentration was correlated with age, BMI, HDL and duration of diabetes in young and middle-aged groups. Plasma PON-1 concentration showed positive correlation with HDL and negative correlation with BMI and duration of diabetes in both young ($p < 0.01$) and middle aged ($p < 0.01$) patients (Figures 3 and 4). However, PON-1 concentration showed no correlation with age ($p > 0.05$) in both groups of diabetic patient. In contrast to our findings Seres et al. [14] showed a negative and significant correlation ($r = -0.38$, $p < 0.0001$) between PON1 activity and age.

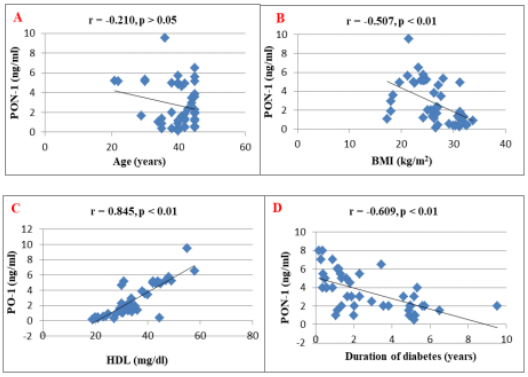

Figure 3. Correlation of plasma PON-1 with age (A), BMI (B), HDL (C) and duration of diabetes (D) among young (30-45 years) patients

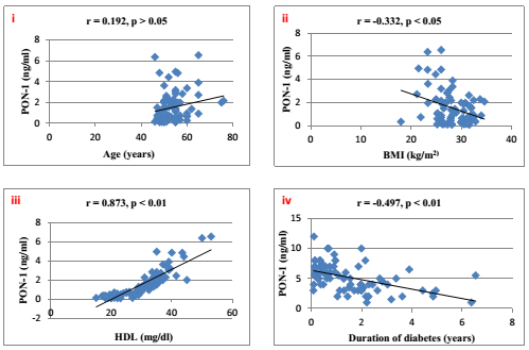

Figure 4. Correlation of plasma PON-1 with age (i), BMI (ii), HDL (iii) and duration of diabetes (iv) among middle-aged (46-60 years) patients

A strong association of PON-1 concentration with duration of diabetes suggests that the enzyme concentration decreases with the progression of disease. The lack of disclosure of diabetes duration in the other study may have been a flaw that tainted the study's findings.

Higher FPG and HbA1c levels in middle-aged diabetic patients showed poor glycemic control in these subjects. Plasma PON-1 concentration also showed a significant and negative correlation with FPG and HbA1c in middle-aged patients (Table 2) and indicates that increasing oxidative stress is linked with deteriorating glycaemic control. Kordonouri et al. [23] also reported a negative correlation between blood glucose levels and PON-1 activity.

Another significant finding of present study is the correlation of PON-1 with BMI in young and middle-aged diabetic patients suggests that having a lower PON-1 concentration might associate with higher risk of obesity. According to a study, the incidence of diabetes increased by 35% for each kg/m2 increase in BMI in the 20-30 year old group and 31% in the 30-40 year old group [24]. It has been observed the diminished levels of PON-1 in obese adults [25]. However, the association between PON-1 and clinical measure of obesity e.g. BMI has only been seen in few investigations [25,26].

5. Conclusion

In conclusion, present work showed a significant reduction in PON-1 concentration in middle aged patients when compared with young patients of T2DM. The diminished PON-1 concentration could be explained by diminished HDL concentration and increasing oxidative stress with aging. Reduced plasma PON-1 concentration showed a strong relationship with other important factors (BMI, HDL and duration of diabetes) that might affect plasma PON-1 concentration with ageing in both young and middle-aged patients suggesting considerable effect on increased risk of obesity and associated disorders which signify the importance of these factors in diabetic patients with ageing.

6. Limitations

Present work did not measure the oxidative stress markers in patients with T2DM. However, the size of the sample is quite enough to draw the meaningful conclusions. Further studies are required to elucidate the mechanism of this reduced concentration of plasma PON-1with ageing.

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References

[1] Salmon, A. B., Richardson, A., & Pérez, V. I. (2010). Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging?. *Free Radical Biology and Medicine, 48*(5), 642-655. https://doi.org/10.1016/j.freeradbiomed.2009.12.015

[2] Kanasi, E., Ayilavarapu, S., & Jones, J. (2016). The aging population: demographics and the biology of aging. *Periodontology 2000, 72*(1), 13-18. https://doi.org/10.1111/prd.12126

[3] Davalli, P., Mitic, T., Caporali, A., Lauriola, A., & D'Arca, D. (2016). ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. *Oxidative medicine and cellular longevity, 2016.* https://doi.org/10.1155/2016/3565127

[4] Ademowo, O. S., Dias, H. K. I., Burton, D. G., & Griffiths, H. R. (2017). Lipid (per) oxidation in mitochondria: an emerging target in the ageing process?. B*iogerontology, 18*(6), 859-879. https://doi.org/10.1007/s10522-017-9710-z

[5] Moya, C., & Máñez, S. (2018). Paraoxonases: metabolic role and pharmacological projection. *Naunyn-Schmiedeberg's archives of pharmacology, 391*, 349-359. https://doi.org/10.1007/s00210-018-1473-9

[6] American Diabetes Association Professional Practice Committee. 2. (2022). Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2022. *Diabetes Care 2022; 45(suppl. 1)*:S17-S38. https://doi.org/10.2337/dc22-S002

[7] Barham, D., & Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst, 97(1151)*, 142-145. https://pubs.rsc.org/en/content/articlelanding/1972/AN/an9729700142

[8] Jeppsson, J. O., Kobold, U., Barr, J., Finke, A., Hoelzel, W., Hoshino, T., ... & Weykamp, C. (2002). Approved IFCC reference method for the measurement of HbA1c in human blood. *Clin Chem Lab Med.2002; Rev.40*: 78-89. https://link.springer.com/article/10.1007/s001250050049

[9] Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *nature, 408*(6809), 239-247. https://doi.org/10.1038/35041687

[10] Jacob, K. D., Hooten, N. N., Trzeciak, A. R., & Evans, M. K. (2013). Markers of oxidant stress that are clinically relevant in aging and age-related disease. *Mechanisms of ageing and development, 134*(3-4), 139-157. https://doi.org/10.2147%2FCIA.S158513

[11] Deakin, S. P., & James, R. W. (2004). Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1. *Clinical Science, 107*(5), 435-447. https://doi.org/10.1042/cs20040187

[12] Goswami, B., Tayal, D., Gupta, N., & Mallika, V. (2009). Paraoxonase: a multifaceted biomolecule. *Clinica Chimica Acta, 410*(1-2), 1-12.

[13] Milochevitch, C., & Khalil, A. (2001). Study of the paraoxonase and platelet-activating factor acetylhydrolase activities with aging. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), *65*(5-6), 241-246. https://doi.org/10.1054/plef.2001.0320

[14] Seres, I., Paragh, G., Deschene, E., Fulop Jr, T., & Khalil, A. (2004). Study of factors influencing the decreased HDL associated PON1 activity with aging. *Experimental gerontology, 39*(1), 59-66. https://doi.org/10.1016/j.exger.2003.08.001

[15] Jaouad, L., de Guise, C., Berrougui, H., Cloutier, M., Isabelle, M., Fulop, T., ... & Khalil, A. (2006). Age-related decrease in high-density lipoproteins antioxidant activity is due to an alteration in the PON1's free sulfhydyl groups. *Atherosclerosis, 185*(1), 191-200. http://dx.doi.org/10.1016/j.atherosclerosis.2005.06.012

[16] Abbott, C. A., Mackness, M. I., Kumar, S., Boulton, A. J., & Durrington, P. N. (1995). Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. *Arteriosclerosis, thrombosis, and vascular biology, 15*(11), 1812-1818. https://doi.org/10.1161/01.atv.15.11.1812

[17] Mackness, B., Mackness, M. I., Arrol, S., Turkie, W., Julier, K., Abuasha, B., ... & Durrington, P. N. (1998). Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. *Atherosclerosis*, *139*(2), 341-349. https://doi.org/10.1016/s0021-9150(98)00095-1

[18] Hedrick, C. C., Thorpe, S. R., Fu, M. X., Harper, C. M., Yoo, J., Kim, S. M., ... & Peters, A. L. (2000). Glycation impairs high-density lipoprotein function. *Diabetologia*, *43*, 312-320. https://doi.org/10.1007/s001250050049

[19] Crow, J. A., Meek, E. C., Wills, R. W., & Chambers, J. E. (2018). A case-control study. The association of serum paraoxonase 1 activity and concentration with the development of type 2 diabetes mellitus. *Diabetes Metab Res Rev, 34*(3).e2967. https://doi.org/10.1002/dmrr.2967

[20] Tavori, H., Aviram, M., Khatib, S., Musa, R., Mannheim, D., Karmeli, R., & Vaya, J. (2011). Human carotid lesion linoleic acid hydroperoxide inhibits paraoxonase 1 (PON1) activity via reaction with PON1 free sulfhydryl cysteine 284. *Free Radical Biology and Medicine, 50*(1), 148-156. https://doi.org/10.1016/j.freeradbiomed.2010.10.708

[21] Khalil, A., Jay-Gerin, J. P., & Fulop Jr, T. (1998). Effect of aging on high density lipoproteins susceptibility to oxidation induced by radiolysis of water. *FEBS Lett*, *435*, 153-158. https://doi.org/10.1016/s0014-5793(98)01058-8

[22] Martínez-Castelao, A., Ramos, R., Gonzalez, M. T., & Castineiras, M. J. (2002). Dyslipidemia and cardiovascular risk in type 2 diabetes mellitus patients with associated diabetic nephropathy. *Nefrologia: Publicacion Oficial de la Sociedad Espanola Nefrologia, 22*, 51-58.

[23] Kordonouri, O., James, R. W., Bennetts, B., Chan, A., Kao, Y. L., Danne, T., ... & Donaghue, K. (2001). Modulation by blood glucose levels of activity and concentration of paraoxonase in young patients with type 1 diabetes mellitus. *Metabolism-Clinical and Experimental, 50*(6), 657-660.https://doi.org/10.1053/meta.2001.23291

[24] Kordonouri, O., James, R. W., Bennetts, B., Chan, A., Kao, Y. L., Danne, T., ... & Donaghue, K. (2001). Modulation by blood glucose levels of activity and concentration of paraoxonase in young patients with type 1 diabetes mellitus. *Metabolism-Clinical and Experimental, 50*(6), 657-660. doi:10.1136/ bmjopen-2018-021768 https://doi.org/10.1136/bmjopen-2018-021768

[25] Ferretti, G., Bacchetti, T., Moroni, C., Savino, S., Liuzzi, A., Balzola, F., & Bicchiega, V. (2005). Paraoxonase activity in high-density lipoproteins: a comparison between healthy and obese females. *The journal of clinical endocrinology & metabolism*, *90*(3), 1728-1733. https://doi.org/10.1210/jc.2004-0486

[26] Bajnok, L., Seres, I., Varga, Z., Jeges, S., Peti, A., Karanyi, Z., ... & Paragh, G. (2008). Relationship of serum resistin level to traits of metabolic syndrome and serum paraoxonase 1 activity in a population with a broad range of body mass index. *Experimental and clinical endocrinology & diabetes, 116*(10), 592-599. https://www.thieme-connect.de/products/ejournals/abstract/10.1055/s-2008-1065350