ORIGINAL ARTICLE

Removal of dental amalgam and other metal alloys supported by antioxidant therapy alleviates symptoms and improves quality of life in patients with amalgam-associated ill health

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Abstract

OBJECTIVES: The purpose of this study was to evaluate treatment of patients suffering from chronic ill health with a multitude of symptoms associated with metal exposure from dental amalgam and other metal alloys.
SETTING AND DESIGN: We included 796 patients in a retrospective study using a questionnaire about symptom changes, changes in quality of life as a consequence of treatment and assessment of care taking.
METHODS: Treatment of the patients by removal of offending dental metals and concomitant antioxidant therapy was implemented according to the Uppsala model based on a close co-operation between physicians and dentists.
RESULTS: More than 70% of the responders, remaining after exclusion of those who had not begun or completed removal, reported substantial recovery and increased quality of life. Comparison with similar studies showed accordance of the main results. Plasma concentrations of mercury before and after treatment supported the metal exposure to be causative for the ill health.
MAIN FINDINGS: Treatment according to the Uppsala model proved to be adequate for more than 70% of the patients. Patients with a high probability to respond successfully to current therapy might be detected by symptom profiles before treatment.
CONCLUSIONS: The hypothesis that metal exposure from dental amalgam can cause ill health in a susceptible part of the exposed population was supported. Further research is warranted to develop laboratory tests to support identification of the group of patients responding to current therapy as well as to find out causes of problems in the group with no or negative results (250 words).

ABBREVIATIONS

ANOVA	Analysis of variance
bcl-2	Anti-death gene
DTT	Dithiothreitol
GABA	γ-aminobutyric acid
GSH	Glutathione, reduced
Hg	Mercury
hSkM1	Gene product of SCN4a (sodium channel a-subunit) being the human homologue of rSkM1, the tetrodotoxin-sensi tive sodium channel characteristic of adult rat skeletal muscle
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IL	Interleukin
IRE	Iron responsive element
IRP-1	Iron regulatory protein 1
MAP	Mitogen activated protein
MELISA®	Memory Lymphocyte Immuno Stimulation Assay
Ras	One of a family of guanosine nucleotide-binding proteins
RIC	Restoration with Individually Compatible Dental Materials
RID	Removal of Incompatible Dental Materials
ROS	Reactive Oxygen Species
TNF	Tumor Necrosis Factor

Introduction

Dental amalgam was used very early in China. "Silver paste" is mentioned in the *materia medica* of Su Kung in 659 A.D. The name used is probably the historical reason why this material in some countries is called "silver amalgam" although its main ingredient always has been mercury. French chemists and dentists experimenting with various mixtures of metals in the end of the 18th century initiated the use of amalgam in the western world. Introduction of dental amalgam is usually ascribed to the French brothers Crawcour in 1831. They used a mixture of mercury and filings of French silver coins. Two years later the Crawcour brothers introduced the filling material in New York and they falsely pretended to be dentists.

Discussions about the rationale in using mercury as the main component in dental amalgams have been going on more than 160 years. In fact, the debate started immediately after the introduction of the material in the U.S.A. American medical-dentists at that time started a merciless crusade against their foreign rivals. They declared that not only was silver amalgam a lousy filling material but it also caused mercury poisoning. This had among other things the consequence that professional dentists started a dental association (The American Society of Dental Surgeons) in New York in 1840 to "increase the standing of the profession and to counteract charlatanry". The first amalgam war had begun.

Almost ignored were the results of studies in which warnings were issued for negative health effects associated with exposure to mercury from dental amalgams [1, 2]. Later during the 1920s, the German chemist Alfred Stock warned about the danger with mercury vapor [3, 4]. As late as in 1939 he issued enhanced warnings [5]. The latest phase in these amalgam wars started in the late 1970s and has been especially intense in Scandinavia but also in the U.S.A. and Germany. Various attempts to estimate risks from dental amalgams have been published advancing conclusions of increased risk of disease [6] as well as no correlation between amalgams and health problems [7]. The latter study, however, demonstrated negligence of combinations of gold and amalgam causing increased corrosion and mercury vapor emission. Richardson [8–10] concludes that a significant portion of all age groups exceeds the proposed reference dose for mercury exposure (0.98 μg Hg/day – tolerable daily intake) more than fives times due to dental amalgam. He also concludes that data suggest that approximately 19 to 20% of the general population may experience sub-clinical central nervous system and/or kidney function impairment as a result of the presence of amalgam fillings. Berlin [11] arrives at the conclusion that the prevalence of side effects from mercury in amalgam on the nervous system, immune system and kidneys should fall in the interval 0.1–10% with the highest probability of 1%. This makes the probable side effects from amalgams a significant health problem.

Documented effects of amalgam removal appeared already in 1842 [1]. However, probably the first comprehensive study was published in 1928 as a consequence of Stock's warnings [15]. Seven patients with a completed treatment reported substantially improved health or complete health. Fleischmann [15] interpreted the symptoms as an expression of "hypersensitivity" and recommended dentistry to abandon copper amalgam of that time immediately and silver amalgam when equivalent materials were available. Several contemporary studies have been published dealing with implications, both in general health, oral pathology and laboratory medicine, of removal of dental amalgam [16–35]. A drawback of most of these studies is, however, that there are few indications of the treatment quality.

The rationale for highlighting clinical effects of chronic low-dose mercury exposure is the advancement of modern research in the behavior of mercury in tissues. This metal has a lot of potentially toxic effects on various levels in a living organism. Mercury exposure decreases the DNA content and increases collagenase-resistant protein formation in synovial tissues. This leads to an increased risk for reduced joint function and decreased ability to repair joint damage [36] partly explaining the joint problems in the patient group.

Decreased amounts of available selenium are also a consequence of exposure to heavy metals, in particular mercury, which compounds the oxidative burden on the body [37]. Mercury also decreases levels of glutathione (GSH) in the body [38]. Mercury binds irreversibly to GSH causing the loss of up to two GSH molecules per mercury ion. The GSH-Hg-GSH complex is excreted via the bile into the feces. Part of the irreversible loss of GSH is due to the inhibition of GSH reductase by mercury, which is used to recycle oxidized glutathione and return GSH to the pool of avail-

able antioxidants [39]. At the same time, mercury also inhibits GSH synthetase, so a lesser amount of new GSH is created. Since mercury promotes formation of hydrogen peroxide, lipid peroxides and hydroxyl radicals, it is evident that mercury sets up a scenario for a serious imbalance in the oxidant/antioxidant ratio of the body [40].

Central nervous affection by exposure to mercury may in part be explained by that Hg^0 and Hg^{2+} are accumulated in motor neurons and Purkinje cells in the brain [41]. Important intracellular effects of mercury are intimately connected to enzymes. All enzymes with sulfur amino acids as well as selenocysteine are open for attack by Hg^{2+} with a probable outcome of impairment of function. Cells presented with Hg^0 will not be able to stop penetration through membranes due to the lipophilicity of uncharged mercury atoms. This opens for a multitude of possible symptoms from various organs in the body. First order thiol binding constant of Hg^{2+} is $10^{30–40}$, which demonstrates the extreme affinity of mercury for thiol groups [42]. Additionally, the ligand exchange rate constant of Hg^{2+} among thiol groups is among the highest known (10^9 s^{-1}), again showing the extreme properties of the mercuric ion [43].

Exposure of workers to 0.0058 mg m^{-3} mercury vapor (0.007–0.021 mg m^{-3}) affected the chemotaxis of polymorphonuclear leukocytes significantly [44]. This exposure is in good agreement to what could be expected from a "normal" set of amalgam fillings [45]. A sensitive subgroup of the population, therefore, has to be expected to suffer from impairment of circulating blood cells. Furthermore, neutrophil activity has been shown to be inhibited by mercury [46]. Even damages to DNA has been attributed to mercury exposure [47].

Mercury interacts with the $GABA_A$ receptor by way of alkylation of thiol groups of cysteinyl residues found in $GABA_A$ receptor subunit sequences [48]. This has the consequence that the binding site of benzodiazepine is modulated.

Structural alteration of the mitochondrial inner membrane with consequent dissipation of membrane potential and disruption of oxidative phosphorylation is another cellular effect of mercuric ions [49–51]. The intracellular calcium homeostasis is altered by mercury inducing mitochondrial release of calcium [51, 52]. Cellular influx of calcium also seems to be a consequence of human exposure to mercury and other metals from dental amalgam [13]. Mercury-induced stress may transform innocuous astrocytes into potentially lethal sources of cytotoxic oxygen free radicals [53].

Low levels of mercuric ions alter the normal pattern of protein tyrosine phosphorylation in B-lymphocytes during antigen receptor-stimulated signal transduction, suggesting that low levels of mercuric ions interfere with signal transduction pathways that are mediated by receptor-associated tyrosine kinases [54]. Additionally, Mattingly et al. [55] showed that low concentrations of mercuric ions interfere with the normal activation of Ras and MAP kinase during antigen receptor-mediated signal transduction in T lymphocytes. The regulation of cell growth is interfered by low and non-toxic levels of ionic mercury [56]. Mercury-induced apoptosis seems to be species dependent in human lymphoid cells in a comparison between the effects of methylmercuric chloride and mercuric chloride [57]. Each of the mercurial species trigger the apoptotic cascade, however, there are profound differences in the mechanism of action at the mitochondrial level. This disparity of mode of action may be linked to differential effects on the anti-death gene, *bcl-2*. Low-dose exposure to silver, copper, mercury and nickel ions alters the metabolism of human monocytes [58]. These authors conclude that the levels of metals released from dental alloys may be significant to monocytic function.

Mercury as well as cadmium binds iron regulatory protein 1 (IRP-1) with high affinity, compared with iron. These metals may cause the disruption of iron metabolism by inhibiting posttranscriptional regulation of iron-related proteins, such as ferritin and transferrin receptor. The effects of these toxic metals on inactivation of IRP-1/IRE binding and activation of aconitase may explain part of the cell toxicity [59]. Even ion channels may be adversely affected by mercury exposure. Divalent mercury blocked human skeletal Na^+ channels (hSkM1) in a stable dose-dependent manner in the absence of reducing agent. Dithiothreitol (DTT) significantly prevented Hg^{2+} block of hSkM1 and Hg^{2+} block was also readily reversed by DTT [60].

Mercury is additionally well known to have adverse effects on the immune system with increased IgE in blood and deposits of immune complexes in the renal mesangium [61, 62]. Immunomodulation is but one of the facets of mercury exposure. Experimental animal studies and observations in humans indicate that immunomodulatory properties of metals such as mercury are heterogeneous and are not restricted to contact allergy [63]. Mercury causes induction of oligoclonal T cell responses skewed toward type-2 reactions [64]. There are numerous studies showing the induction of autoimmunity by mercury exposure [65–69]. Even neurological diseases have been hypothesized to be, at least partly, due to induction by exposure to heavy metals such as mercury [70–72].

During the last twenty years an increasing number of patients have sought dental and/or medical care for problems possibly associated with dental amalgam. These patients have observed a relationship in time between odontological treatment and occurrence or increase of their symptoms. The metal syndrome was conceived by our group as a collective term describing such patients with a series of symptoms for which no other etiologic diagnosis could be found in spite of thorough examination and laboratory tests [12]. Most other possible causes, except for metal exposure from amalgams, for the disease of these patients have been excluded by meticulous investigations performed by several specialist physicians. A differential diagnostic

procedure had thus been thoroughly implemented. These patients suffered from several general, neurological, psychiatric and oral symptoms.

Soon additional laboratory tests were included in these studies. Nuclear microscopy of single isolated blood cells revealed that patients, in contrast to healthy controls, displayed distorted profiles of trace elements in blood cells [13]. In addition, changes of trace elements in blood plasma assessed by X-ray fluorescence were observed.

Hypersensitivity or allergy to metals comprising dental alloys was suspected rather early. To avoid potential side effects of traditional patch testing, an *in vitro* test was applied. This test being called MELISA® (MEmory Lymphocyte Immuno Stimulation Assay) is a development of the common lymphocyte transformation test. MELISA® applied to 3000 patients with suspected side effects from metals in dental restorative materials in three analytical centers demonstrated a reasonable degree of conformity [14].

The aim of the present study was to evaluate the treatment of patients at the former Department of Clinical Metal Biology at the University Hospital in Uppsala, Sweden. A questionnaire comprising questions about symptoms, quality of life as well as care-taking assessment was constructed and sent to patients. The study design was, therefore, a before-after design in which patients constituted their own controls and was undertaken in retrospect and longitudinally. This design has the attractive advantage of setting aside genetic differences between cases and controls. Even if the study had been prospective, it would not have been possible to adopt a double-blind placebo-controlled design for obvious reasons.

Patients and methods

Characterization of patients

During the period from 1991 to 1996 about 1000 patients were investigated out of 2000 patients referred to the Department of Clinical Metal Biology, of which about 50% from the County of Uppsala. The present evaluation comprises 796 patients that were medically examined until October 1996. The patients resident in the County of Uppsala were referred either by physicians or dentists, whereas patients from other parts of Sweden were referred by physicians only. It was not possible to influence the selection of the patients by the Department of Clinical Metal Biology. The department was commissioned to improve care taking and to develop diagnostic procedures and treatment. Patients were admitted to the therapy on a regular basis after routine referral procedures. All patients gave an informed consent.

All patients had experienced chronic or long-lasting disease and severe sufferings. These problems had resulted in a great number of futile contacts with health care institutions. In this way, they had constituted a "wandering queue" of patients with a low quality of life causing high costs for the society comprising medical care, drugs and sick-leave as well as early retirement.

The majority of the patients were 45–60 years of age with more than two-thirds women. In this report 123 men (mean age 51 years, range 19–82 years) and 340 women (mean age 53 years, range 22–82 years) are included.

One early experience in examining the patients was that they had been handled in a qualified way in the health care system even though no etiologic diagnoses were arrived at. No serious non-treated diseases of other origin were discovered.

Consequently, the patients had been through extensive medical examinations without a nosological description of the symptoms in the majority of the cases. A small part of the patients, however, had been diagnosed with rheumatological or neurological diseases. The multitude of symptoms displayed by these patients or concomitant worsening on dental treatment nevertheless raised suspicion of adverse effects on the health from dental materials. These patients were, therefore, not excluded from continued examination at the department.

Case history of the patients

As a primary basis of a diagnosis an extensive anamnesis was recorded as well as earlier contacts with health care institutions. This was complemented with comprehensive enquiries to facilitate future systematic evaluations.

The pattern of medical problems in this patient group is complex. It involves many parts of the body with symptoms such as ambulatory musculo-skeletal pains, disturbances of the function of the gastro-intestinal system, hormonal perturbations as well as neuropsychological symptoms. Characteristic features of the patient group are a multitude of symptoms and that subgroups with different distribution of the predominant symptoms seem to exist. The dominating initial symptom is chronic fatigue. A general pathological process, without infectious or oncological genesis, seems to be characterizing the polysymptomatic situation of the patients. Infectious or oncological etiology of the symptoms had already been excluded before the patients were referred to the department.

Exposure anamnesis

In this the patients' contacts with metals, mould, solvents and other chemicals in the environment, both occupational and resident, were registered. Also metals and other components of dental materials were considered. This work is rendered more difficult because dental materials have a complex composition. Furthermore, these materials are classified as medical technical products for which there are no requirements of detailed declaration of the content. Dental amalgam is usually comprised of 3–4 metals with mercury as the dominating ingredient. Dental alloys with gold are always composed of numerous precious and base metals. White dental restorative materials, for example composite resins, contain a number of compo-

nents that are potentially allergenic. All these materials release compounds that can be inhaled and/or swallowed with a varying degree of uptake in organs. The amount released depends on numerous factors and it varies from one individual to another and this also accounts for the final uptake in the human body. Furthermore, the individual sensitivity to this exposure strongly varies as well as the potential symptoms.

From the comprehensive anamnesis it was often clear that the patients had experienced adverse effects expressed as aggravating symptoms in relation to dental treatments. Some of them also reported skin problems from contact with metals such as jewelry and jeans buttons. These facts substantiated the suspicion of adverse effects in the form of immunological reactions caused by exposure to metals.

Somatic examination

A customary somatic examination was performed and in addition the various types of dental restorative materials in the patient's mouth were registered together with their status.

Routine blood samples

A panel of routine blood tests was performed as part of a general check of the health status and to acquire start values to be used for a continued systematic evaluation.

Determination of trace elements in blood plasma

Venous samples were drawn in metal free vacuum tubes, at the same time of the day, before and after treatment. After separation of erythrocytes and blood plasma both fractions were frozen and stored at –86°C until analysis. For the purpose of monitoring possible changes of the mercury concentration between the sampling points, a random set of 165 samples (before and after treatment) was selected for analysis of mercury in blood plasma. The samples were decomposed in high-purity grade 65% nitric acid (Scandchem, Norway) with a very low contamination of mercury and other metals. Decomposition was performed in quartz tubes put into steel bombs. These were sealed with the same momentum and put in an oven at 180°C for four hours. Thereafter, an internal standard of indium was added and the samples diluted with ELGA-water (>18 MΩ-cm) to an appropriate volume. The mercury analysis was performed in an inductively coupled plasma mass spectrometer (ICP-MS, Perkin-Elmer Elan 6000) and the quality control was assessed by certified reference materials (Seronorm, Nycomed, Norway) randomly allocated to be in average every fifth sample. Accuracy and precision was <5% and <4%, respectively.

Special tests

Routine laboratory tests usually do not present any diagnostic guidance as far as side effects of dental materials are concerned. According to the commission from the Board of the County Council, new ways were to be sought to indicate adverse effects of metal exposure. By performing special tests valuable information was gathered, which hopefully will lead to increased certainty in the diagnostics. Results from special tests such as nuclear microscopy for determination of trace-element profiles in blood cells as well as *in vitro*-tests of metal hypersensitivity (MELISA®) will be published and discussed elsewhere. Examples of results from such methods in conjunction with metal exposure can be found in Lindh et al. [13] and Stejskal et al. [14], respectively.

Treatment

Based upon results from the extensive examination of the patients it turned out to be reasonable to avoid, as far as possible, continued exposure to the incriminated metals of dental restorative materials. This recommendation is founded on the general precautionary principle of allergists that an individual should avoid compounds possibly causing allergy or hypersensitivity. There were no indications for any other medical treatment but reduction of the metal exposure in the majority of the cases and supplementation with suitable antioxidants and, where, appropriate, immunomodulating therapy to counteract side effects during dental treatment.

Medical treatment

After diagnostic work up and evaluation each patient was put on a standard regimen of anti-oxidants orally. These included:

- Vitamin C 1900 mg/day
- Vitamin B-complex: B1 30 mg/day, B2 30 mg/day, Niacin 150 mg/day and B6 6 mg/day, pantothene 30 mg/day
- Vitamin E 400–600 mg/day
- Sodium selenite 400 μg/day

The antioxidant formulas were standard products available at Swedish pharmacies and were prescribed with regular health care benefits for the patients.
In cases with positive markers of vitamin B12-deficiency in the cerebrospinal fluid additional treatment with B12 and Folic acid was prescribed:

- Methyl cobalamin 5 mg/ml, 2 ml/amp, for s.c. injection 1 amp/week).
- Folic acid 10 mg/day

Dental treatment

After completion of medical examination patients were, according to occurring indications, recommended a removal of incriminated dental materials. Dental amalgam was found the most offending dental material, followed by different gold alloys and non-precious metal alloys, e.g. titanium alloys or chromium-vanadium alloys. The aim of the dental treatment was to remove all manageable amounts of these materials, detectable by dental investigation methods, e.g. x-rays.

Soon, in the course of the dental treatment, it became clear that symptom aggravations occurred almost uniformly after removal of incriminated materials or after the introduction of certain replacement materials. The typical time delay of these reactions supported the original contention that hypersensitivity to the dental materials was at least one cause of the patients' ill health.

It appeared clear that a systematic and intimate cooperation between physicians and dentists was necessary to handle sometimes-dramatic changes in the patients' symptom complex during dental treatment.

The fact, that many patients were referred from other parts of the country made it practical for them to consult their regular dentists for the ensuing treatment. However, about half the patients were referred to a few dental clinics in Uppsala County, which have put precautionary methods into practice to protect the patients from unnecessary large exposure to offending substances, which inevitably occurs in routine dental treatment. Especially patients considered severely sensitive were referred to such clinics.

Based on clinical experiences, a protocol for the removal of incompatible dental materials (RID) and bite restoration with individually compatible materials (RIC) was implemented. Adequate equipment in the working premises e.g. catalytic mercury vapor traps and high-volume-suction-capacity equipment was mandatory. Further, detailed instructions for the use of specified burrs, diamond bits or ultra-sonic bits for every type of metal restoration were followed. Also instructions for the use of different barriers between patients' teeth and adjacent tissues in the oral cavity are part of this protocol.

For the patients who showed intolerance reactions to replacement materials of non-metallic type such as dental composites, cements, root canal materials etc., a clinical trial system for the choice of individually compatible dental restorations was developed.

Most biocompatible were different ceramic materials together with individually chosen dental cements. The recent improvement of mechanical properties of such materials now allows bite restorations with non-metallic materials in almost all clinical cases. The application of modern odontological techniques also allows such treatment to be performed in a lenient way concerning both the effect on dental tissues and the patients personal experience of the whole dental treatment.

Questionnaire

Evaluation of the treatment results was based on a questionnaire that was constructed in co-operation with a group of scientists from the Medical Faculty at Uppsala University and with representatives also from the universities in Umeå and Lund. Of the 1000 treated patients, 796 were estimated to be homogeneous as to the treatment protocol and the questionnaire was sent out to them. Participation in the retrospective study was voluntary and anonymous. The questionnaire was composed of five different categories of questions, three of which are included here. The symptoms included in the questionnaire were selected to represent the most common from clinical experiences of the patient group (cf. Appendix). Thirteen of the symptoms were emphasized because they had appeared in earlier studies of amalgam removal [16, 22].

To efficiently extract information from the answers in the questionnaire, an improvement score was constructed. Severity of symptoms was estimated on a scale from 1 to 5, where 1 represents "no troubles" and 5 "serious troubles". A first part of the improvement score comprised the difference in severity between before and after. Maximum improvement then is 4 and maximum worsening is –4. The second part comprised the estimation of symptom change, which was done on a scale 1–7, where 1 represented "much better" and 7 "much worse". Score 4 was equivalent to "no change". To be compatible with the first part, the scale was changed so that "No change" was given score 0, "much worse" score –3 and "much better" score +3. These two parts were added and constituted the improvement score with a maximum of 7 and a minimum of –7.

Statistical methods

Wilcoxon's signed rank test was used to compare before and after RID situations because skewed distributions were expected. For the comparison of response profiles in the answers before RID, a χ^2 test with partitioning of the χ^2 was used. This technique resembles ANOVA with subsequent multiple comparisons.

Results

Of the targeted 796 patients, 513 responded with a returned questionnaire. The relatively high non-response (35.6%) prompted an investigation of non-responders in co-operation with Statistics Sweden. This showed that non-responders did not constitute a group separate from the responders.

Based upon information given by the patients, 50 were excluded from the continued analysis because they did not begin or complete the RID procedure during the study time. Thus, in the following results, 463 individuals are represented.

A priori hypotheses about symptoms that could be expected to improve from the treatment were not possible to formulate. The median number of the 30 symptoms experienced by the patient group was 19 and 6.9% had all 30. The symptom frequencies before RID regarding all patients are shown in Table 1. Symptom numbers refer to the list in the Appendix.

Straightforward analysis of symptom changes from before RID to after RID by Wilcoxon's signed rank test gave the result that all thirty symptoms were significantly ($p<0.01$) reduced in the entire group of patients. This analysis, however, concealed variations between subgroups of individuals. Therefore, the results from the questionnaire about quality of life were used as the

basis for further analysis and presentation.

Quality of life

Figure 1 shows the change in the total quality of life measured as the difference in scores before and after RID. The histogram comprising 463 individuals makes three groups of patients evident. A change of one unit or more was considered a true difference. A majority of the patients (n=334, 247 women, median age 53 y, range 22–76 y; 87 men, median age 52 y, range 26–76 y) belong to the greatest group for which there is a positive change in the quality of life. This group will be denoted the positive group. Another group (n=69, 49 women, median age 54 y, range 23–76 y; 20 men, median age 53 y, range 19–76 y) with a zero difference in the quality of life will be denoted the zero group and the last group (n=60, 44 women, median age 55 y, range 30–82 y; 16 men, median age 45 y, range 26–71 y) with a negative difference in the quality of life is analogously denoted the negative group.

Symptom changes

Further treatment of data and their presentation is based upon the three groups established using quality of life information. There are four different measures of symptom changes. The first is the frequency of symptoms, the second is the severity of symptoms as estimated by the patients and the third is the total number of symptoms. Data on severity of symptoms is further elaborated into an improvement score being the fourth.

Frequency of symptoms

Figure 2 shows the frequency of symptoms before, during and after RID in the positive group. Table 2 displays the central tendency of the symptoms frequencies in the negative and zero groups.

Positive group

Most symptoms in this group remained unchanged in frequency during RID or only small changes were observed with a tendency to increased

Table 1: Symptom frequencies before RID in the entire group. Numbers refer to the symptom questions in the questionnaire (cf. Appendix).

No	Symptom	Frequency (%)
1	Chronic or periodic fatigue	74.5
17	A feeling of dejection or depression from time to time	74.3
6	Pain or discomfort in the muscles	73.7
7	Abnormal fatigue after physical exertion	72.4
16	Impairment of concentration	72.1
5	Muscle discomfort in the whole body	70.2
25	Troubles with stomach/intestines	69.8
29	Blisters, wounds or other discomfort in the mouth	69.5
18	Impairment of sleep	67.8
21	Discomfort in hands/feet	67.8
12	Impaired memory (forgetfulness)	67.6
19	Dizziness or unsteadiness	64.6
8	Headache	62.6
15	Difficulties to think	62.0
9	Troubles in the joints	61.6
28	Often infections	61.3
23	Aching shoulder/shoulders	60.7
10	Light sensitivity	60.3
13	Overly irritated	60.0
2	Feeling cold, shivering or fever	59.6
30	Aching teeth, jaws or face	57.9
3	Sore throat, other throat problems	55.1
22	Tremor or spasms in muscle/muscles	54.6
24	Troubles with the heart	53.8
26	Troubles with the urinary bladder/urinary production	49.0
14	Experience of bewilderment	47.9
27	Eczema, blisters or other skin troubles	47.1
11	Temporary impairment of vision	43.4
20	Buzzing in the ears (tinnitus)	40.4
4	Aching lymphatic glands on the neck or in armpits	37.6

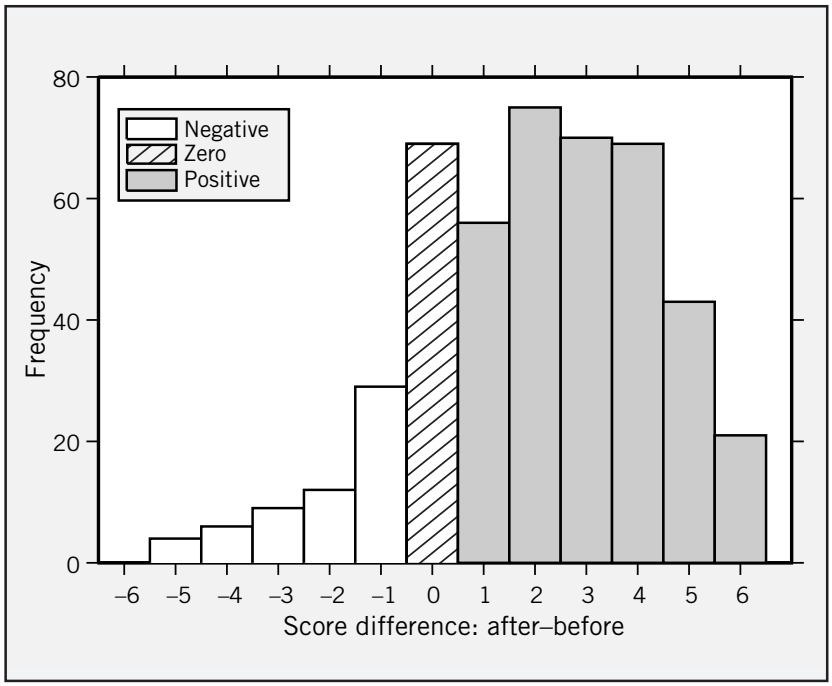

Figure 1. Differences in estimations of the quality of life before and after RID. Score 1–7 corresponding to "*very bad*" – "*very good*".

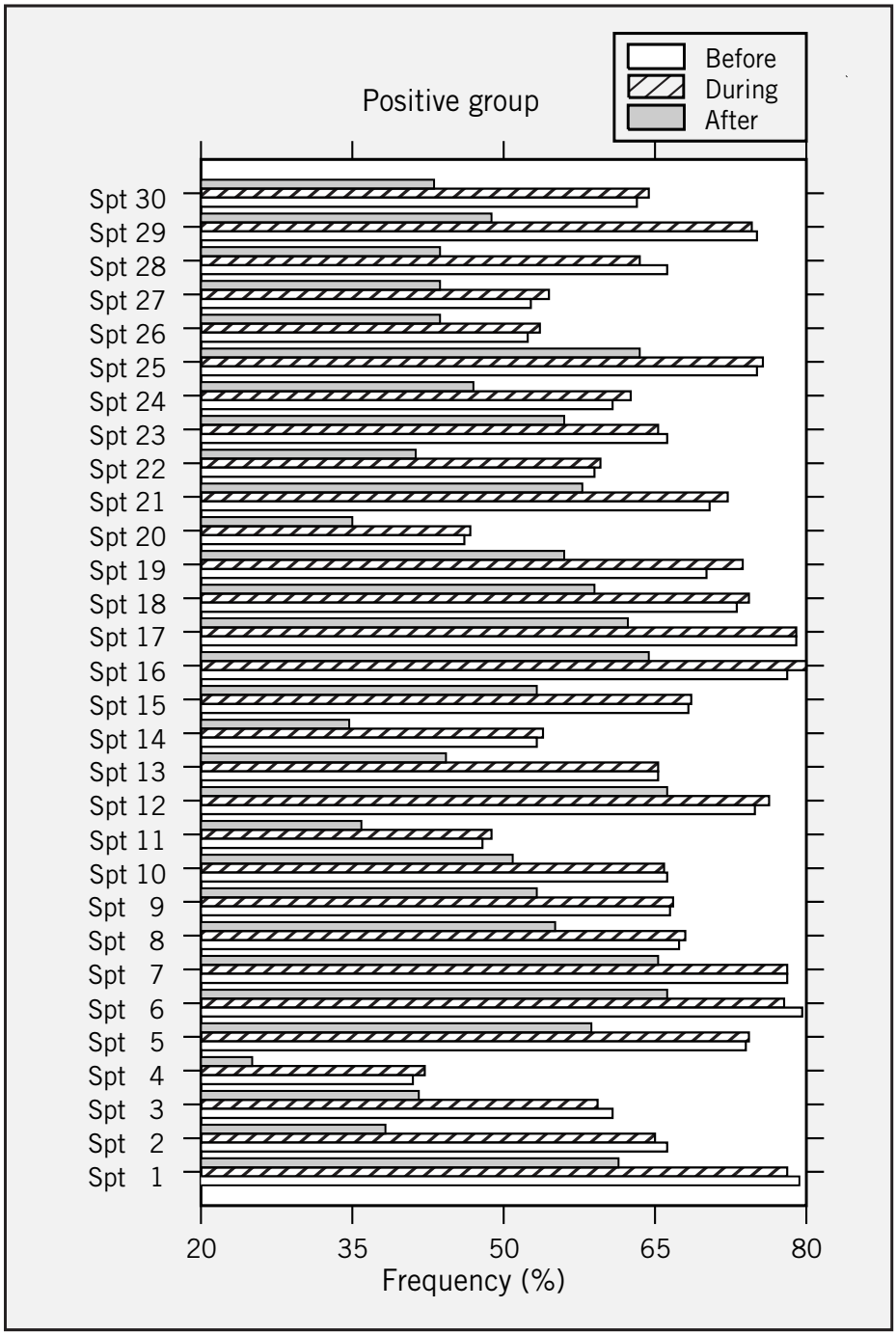

Figure 2. Symptom frequencies before, during and after RID in the positive group.

frequency. Small decreases were also found for a few symptoms. However, after RID there was a significant decrease. In the positive group symptom 6 "pain or discomfort in the muscles" was the most common. It remained the most common after RID.

Negative group

In this group, frequencies increased for all symptoms during RID except for symptom 28 "often infections". The overall change of frequency between before and after was an increase. Exceptions are symptom 4 and 13 corresponding to "aching lymphatic glands" and "overly irritated" for which there was no change. Symptoms 28 and 29 corresponding to "often infections" and "blisters etc. in the mouth" showed decreased frequency.

The tendency of frequency change in the negative group was a substantial increase during RID and a decrease after RID. The decrease however did not reach the level of before treatment. In the negative group, the most common symptom before RID was symptom 1 "chronic fatigue". It remained the most common after RID.

Zero group

Frequencies increased during RID for most of the symptoms in the zero group except for symptoms 1, 9, 15, 19, 23, 27 and 29 corresponding to "chronic fatigue",

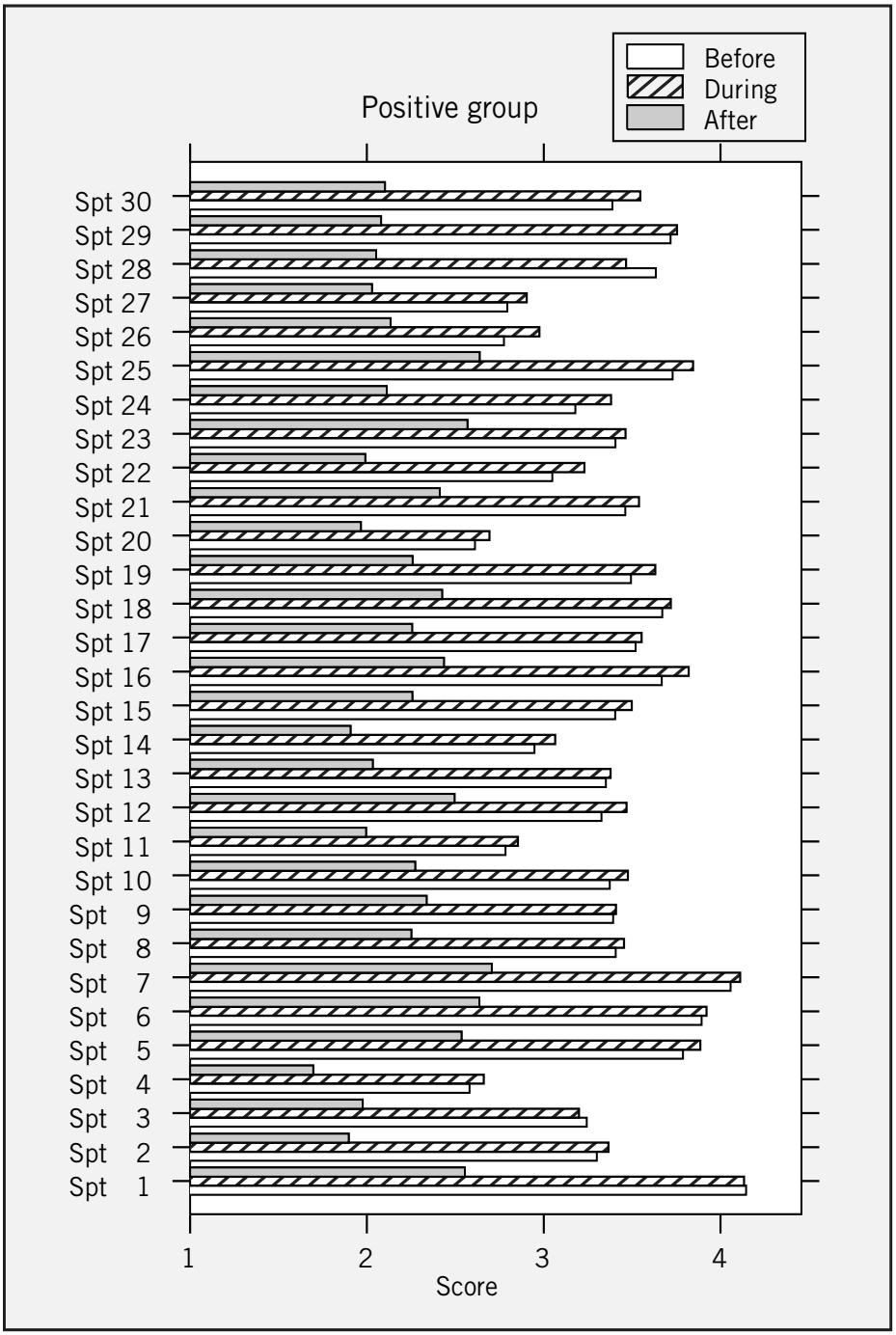

Figure 3. Estimations of symptom intensity (severity) before, during and after RID in the positive group. Score 1–5 corresponding to "*no troubles*" – "*severe troubles*"

"troubles in the joints", "difficulties to think", "dizziness or unsteadiness", "aching shoulders", "eczema etc." and "blisters etc. in the mouth" for which no difference was demonstrated. Another notable exception was that symptom 28 "often infections" decreased in frequency during RID. Unchanged frequency was observed between before and after RID for symptoms 3, 9, 14 and 17 corresponding to "sore throat etc.", "troubles with the joints", "light sensitivity", "bewilderment" and "dejection or depression". Symptoms 6, 16, 27, 28 and 29 corresponding to "pain or discomfort in the muscles", "impairment of concentration", "eczema etc.", "often infections" and "blisters etc. in the mouth" exhibited lower frequency after RID. In the zero group the difference in frequency between before and after was very small and the increase during RID rather moderate. The most common symptom in the zero group was symptom 17 "dejection or depression". It was exchanged by symptom 25 "troubles with stomach/intestines" after RID.

Severity of symptoms and improvement scores

Figure 3 shows the assessment of the severity of the symptoms before, during and after RID. The scores were on a 5-level scale from 1 to 5 and 1 being no troubles and 5 severe troubles. The central tendency of symptom severity in the negative and zero groups are shown in Table 3. Table 4 displays results of the statis-

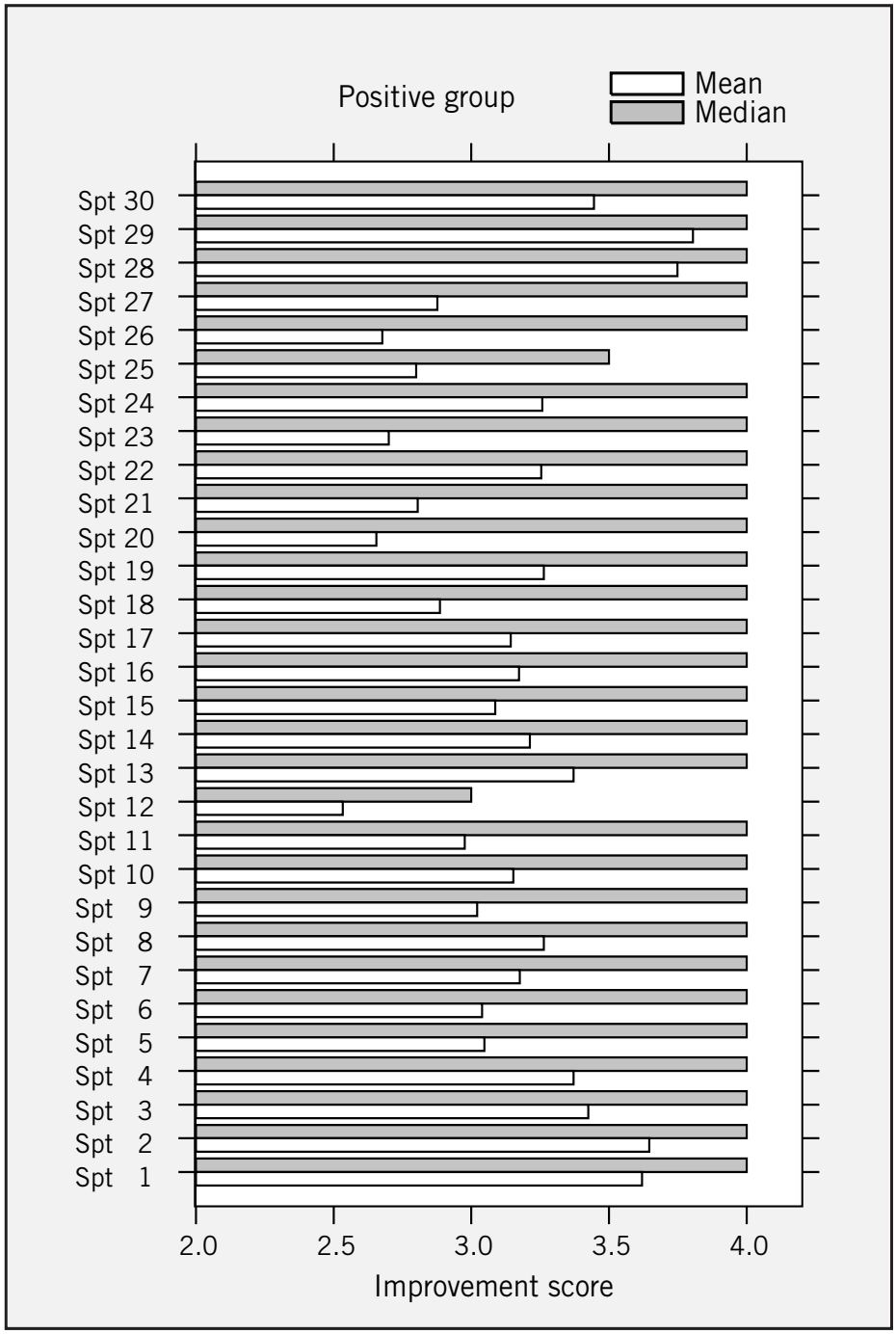

Figure 4. Improvement scores in the positive group. Score –7 to +7, where +7 is the best improvement.

tical analysis of symptom differences before and after treatment. Figure 4 displays the mean and median improvement scores for the positive group and the 30 symptoms. The central tendency in the negative and zero groups are displayed in Table 6.

Positive group

This group usually displays a higher initial assessment and a greater difference between before and after values than the other groups. After RID all symptoms are reduced significantly in severity. From Figure 5 it can be seen that, with only a few exceptions, the median improvement score is 4. Symptoms for which there were no improvement in the negative and zero groups in contrast to score 3 or 4 in the positive group include 1, 7, 12, 18 and 21 representing "chronic fatigue", "fatigue after physical exertion", "impaired memory", "impairment of sleep" and "discomfort in hands/feet". Score 4 was given in all three groups for symptoms 4 and 28 representing "aching lymphatic glands" and "often infections".

Negative group

The initial values are about the same as in the zero group. In the negative group there is a substantial deterioration during RID and the scores do not return

Table 2: Central tendency of symptom frequencies before, during and after RID in the negative and zero groups.

	Negative (%)			Zero (%)		
	Before	During	After	Before	During	After
Mean	49.6	61.4	57.2	45.0	46.9	47.2
Median	48.3	62.5	56.7	44.2	47.1	47.1

Table 3: Central tendency of symptom intensity (severity) before, during and after RID in the negative and zero groups.

	Negative			Zero		
	Before	During	After	Before	During	After
Mean	2.8	3.4	3.0	2.7	2.9	2.6
Median	3.0	3.5	3.0	3.0	3.0	3.0

Table 4: Results of Wilcoxon's signed rank analysis of symptom differences before-after.

Symptom	Positive	Zero	Negative	Symptom	Positive	Zero	Negative
1	<0.001	NS	NS	16	<0.001	NS	NS
2	<0.001	NS	NS	17	<0.001	NS	NS
3	<0.001	NS	NS	18	<0.001	NS	<0.05
4	<0.001	NS	NS	19	<0.001	NS	NS
5	<0.001	NS	<0.05	20	<0.001	NS	NS
6	<0.001	NS	NS	21	<0.001	NS	NS
7	<0.001	NS	<0.05	22	<0.001	NS	NS
8	<0.001	NS	NS	23	<0.001	NS	NS
9	<0.001	NS	NS	24	<0.001	NS	NS
10	<0.001	NS	NS	25	<0.001	NS	NS
11	<0.001	NS	NS	26	<0.001	NS	NS
12	<0.001	NS	<0.01	27	<0.001	NS	NS
13	<0.001	NS	NS	28	<0.001	<0.05	<0.01
14	<0.001	NS	NS	29	<0.001	<0.01	NS
15	<0.001	NS	NS	30	<0.001	NS	NS

NS: $p \geq 0.05$, not significant

Table 5: Number of symptoms before and after RID in all groups.

	Total (n=463)		Positive (n=334)		Zero (n=69)		Negative (n=60)	
	Before	After	Before	After	Before	After	Before	After
Mean	18.2	15.2	19.8	14.7	13.5	14.1	14.9	19.0
Median	19	15	22	14	14	13	16	19.5
30 spt	32	10	28	6	1	1	3	3
30 spt (%)	6.9	2.2	8.4	1.8	1.4	1.4	5.0	5.0

Table 6: Central tendency of improvement scores in the negative and zero groups.

	Negative	Zero
Mean	1.3	1.6
Median	2.0	2.0

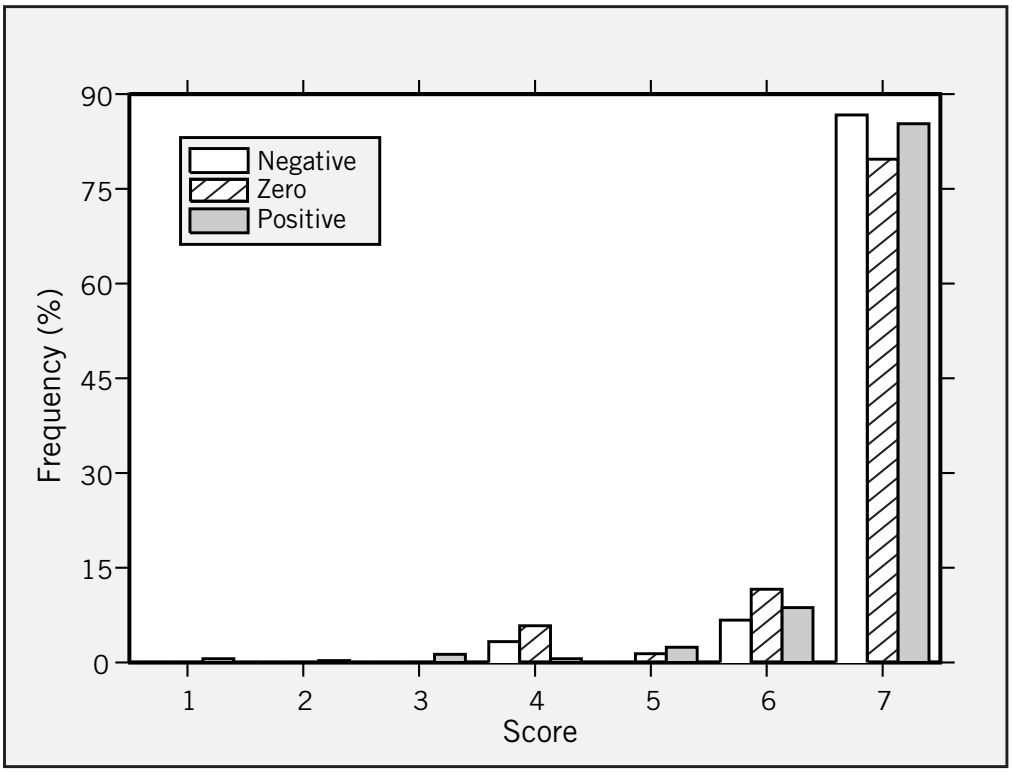

Figure 5. Assessment of the activities and care at the Department of Clinical Metal Biology in the negative, zero and positive groups.
Score 1–7 corresponding to "*very bad*" – "*very good*".

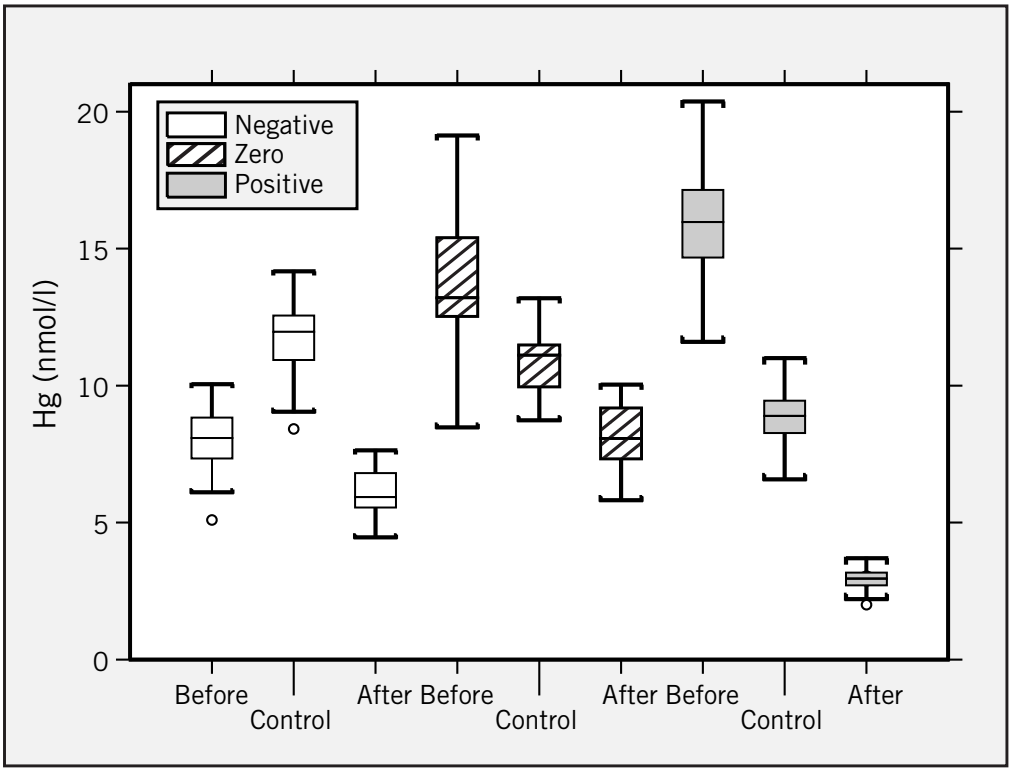

Figure 6. Mercury concentrations in blood plasma before and after RID in the negative, zero and positive groups. For each group, the values of matched control groups (healthy individuals) are inserted.

Table 7: Results from a χ^2 test of homogeneity of response profile concerning symptom intensity before RID.

Symptom	χ^2	*p*-value	Symptom	χ^2	*p*-value
1	28.952	0.0013**	16	38.848	$2.6922 \cdot 10^{-5}$***
2	28.745	0.0014**	17	29.452	0.0011*
3	25.503	0.0045**	18	30.445	0.0007***
4	15.789	0.1058	19	36.190	0.0001***
5	29.143	0.0012**	20	22.769	0.0116*
6	36.938	0.0001***	21	17.225	0.0695
7	38.375	$3.2640 \cdot 10^{-5}$***	22	21.453	0.0181*
8	29.162	0.0012**	23	25.182	0.0050**
9	18.957	0.0408*	24	31.475	0.0005***
10	24.447	0.0065**	25	50.968	$1.7698 \cdot 10^{-7}$***
11	15.886	0.1029	26	16.586	0.0840
12	39.179	$2.3611 \cdot 10^{-5}$***	27	24.480	0.0064**
13	28.469	0.0015**	28	29.070	0.0012**
14	19.648	0.0328*	29	23.797	0.0082**
15	30.103	0.0008***	30	26.209	0.0035**

*** = $p<0.001$. ** = $p<0.01$. * = $p<0.05$

Table 8: Comparisons between the present study and two other studies with similar symptoms in the questionnaire. Numbers given are percentages of total improvement of the symptoms. The second figure is the present study.

Symptom	Olsson & Lindh [22]			Klock et al. [16]		
	Better	Equal	Worse	Better	Equal	Worse
Chronic fatigue	30/24					14/24
Pain or discomfort in muscles	30/18			23/18		
Headache	51/29					31/29
Troubles in the joints	32/29					
Impaired concentration	27/23					9/23
Dejection or depression	29/26			37/26		
Aching shoulder/shoulders		24/25				
Heart troubles			33/37			
Troubles with stomach/intestines	34/20					
Eczema, blisters or other skin troubles		39/39				
Blisters, wounds in the mouth	57/38					
Aching teeth, jaws or face		43/41				

to the initial values. There is a significant worsening of symptoms 5, 7, 12 and 18 corresponding to "muscle debility", "fatigue after physical exertion", "impaired memory" and "sleep disturbances". For the symptom 28 "often infections", however, there was a significant reduction of the complaints. From Table 6 it can be seen that the central tendency for this group is not very different from that of the zero group. However, there are more frequent improvement score 0 compared to the zero group.

Zero group

The initial values are about the same as in the negative group. There is a tendency to experience a relief in the zero group, however, at least for several of the symptoms. A significant relief is noted of symptoms 28 and 29 corresponding to "often infections" and "blisters etc. in the mouth". From Table 6 it can be seen that there are more frequent improvement score 4 compared to the negative group but that the central tendency is very similar.

Total number of symptoms

The total number of symptoms before and after RID is shown in Table 5. There is a similar trend in the changes of the total number of symptoms as in the assessment of severity.

Assessment of care taking

Figure 5 shows how the patients in the three different groups assessed the activities and care at the Department of Clinical Metal Biology. The pattern of assessment is rather similar in the groups. In fact, the negative group displayed the highest fraction in the score 7 (89.7%) compared to the positive (86.2%) and zero groups (81.1%).

Heterogeneity before RID

Clinical experience from these patients led to the hypothesis that the group was not homogeneous but separated in sub-groups with different main points of the symptomatology. To investigate the possibility of heterogeneity of symptoms among the patients before RID, the response profile in symptoms a χ^2 test with partitioning of χ^2 was undertaken. The symptom intensity was estimated on a scale from 1 to 5, where 1 corresponds to "no troubles" and 5 to "severe troubles". Some of the patients did not report their estimates for some of the symptoms. This partial non-response cannot, however, be neglected and the frequency of non-response was included in the analysis. Results of this test of response profile are presented in Table 7.

It is obvious that the patient group was not homogeneous in this respect. Only for the questions 4, 11, 21 and 26 corresponding to the symptoms "aching lymphatic glands", "temporary impairment of vision", "discomfort in hands/feet" and "troubles with the urinary bladder/urinary production" the group was homogeneous. The partitioning of χ^2 also showed that the positive group differs from the other groups except for the symptoms mentioned above. It was not, however, possible to differentiate between the negative and the zero groups.

Comparison with similar studies

Two earlier studies of amalgam removal were based on a similar questionnaire. All but one of the symptoms in the earlier studies was included in the present investigation. It was not possible, however, to compare all of the symptoms due to lack of presentation in one study. Table 8 presents the results of a comparison as far as it has been possible. The focus was on total improvement of the symptoms and percentages of such results are compared in Table 8. Klock et al. [16] reported results only for six symptoms, one of which was "burning mouth or metal taste" not being included in the present study. This symptom was included also in the study by Olsson and Lindh [22].

Quality of life versus mercury concentration in blood

To investigate how treatment of the patients was related to the mercury concentration in blood plasma three randomly selected sets of patients were sampled. From the positive group 100 blood samples representing the situation before and after RID were assessed for the mercury concentration in blood plasma. From the zero and negative groups 35 and 30 samples, respectively, were assessed in the same way.

For comparison, three sets of control blood plasma were sampled as closely as possibly matched to the patient subgroups regarding age, gender and socio-economic situation. Subjectively healthy persons not suffering from any disease and lacking diagnosis as well as not being on any medication comprised the control groups.

Results from measurements of mercury concentration in blood plasma with ICP-MS are presented in Figure 6. This figure is a box-plot diagram in which the upper lines of the boxes represent the upper quartile while the lower lines represent the lower quartile. The whiskers extend out to maximum and minimum values if the distance does not exceed 1.5 times the interquartile range. In such cases, a circle denotes extreme values.

The positive group had before values that are higher than both other groups. Additionally, the after values of the positive group are substantially lower than any of the other groups including the three control groups. In fact all differences between before values in all groups were significant ($p<0.001$). Even the after values were significantly different ($p<0.001$) between all groups (Figure 6).

Discussion

The present study is to our knowledge the first based on a development of diagnostic methods and improvement of treatment in a consequent co-operation between physicians and dentists. The main results demonstrate that the Uppsala model appropriately handles diagnosis and treatment in more than 70% of the patient group.

Trace elements, most of which are metals, play important roles in biology. Some 15 are generally considered to be essential for life [73]. These include manganese, iron, nickel, copper and zinc, whereas important non-metals are selenium and iodine. The beneficial effects of the essential trace elements are exerted within often-restricted dose limits in intervals usually called safe and adequate intake. Too low intake results in deficiencies and too high intake in toxicities. The question of toxicity is thus only one of dose. Metals included in dental amalgams, especially mercury, silver and tin, are not considered to be essential for life. On the contrary, they are known mostly for their adverse effects on living organisms [74]. Even precious metals like gold and platinum, by some considered biologically inert, have known pharmacological effects [75, 76]. It

is, therefore, prudent to expect them to have biological effects even at lower doses.

Cumulative and collective effects may well explain the plethora of symptoms experienced by patients with ill health associated with exposure from the components of metallic dental restorative materials. Mercury is well known as a potent toxin producing various symptoms from several organ systems [77]. It is not surprising, therefore, that most of these patients complain about a series of symptoms, many of which could be explained by various known diseases. Factors able of affecting several organs at the same time could be, among others, of toxic or immunological origin. The adverse effects of metals like mercury are thought mainly to be reactions with sulfur-containing groups in proteins [78, 79] being in accord with affection on a multitude of biological systems.

The basis for all such negative health influence of metals in dental restorations is their bioavailability. Metals used in dental materials are released in the oral cavity in amounts far from negligible. It is now generally accepted that all metal alloys, especially amalgams, are unstable and release varying amounts of metals continuously. The corrosion process, especially when different metals are present, promotes the release of metal ions and mercury vapor. The daily release of metals in the oral cavity is certainly high enough to cause immunological reactions in sensitive individuals since, theoretically; only a few atoms of an offending metal might be necessary.

Affection of the immune system may thus be important. Such effects could be either direct toxic action on the immune system or reactions triggering hypersensitivity or allergy. Nickel is a metal recognized to be essential at least for bacterial systems [80] and is responsible for a significant health problem of allergy. The prevalence among women may be as high as 38%, whereas in men it is perhaps only 3% [81]. Difference between genders in sensitivity to metals might be one of the factors explaining the dominance of women in the present patient group. Even mercury is immunotoxic [82] and causing hypersensitivity [83]. Also gold and titanium have considerable effects on the immune system causing hypersensitivity [14, 84, 85]. It is concluded that there is a positive relationship between contact allergy to gold and presence and amount of dental gold alloys [84].

Titanium is since decades considered to be a metal with many biologically useful properties which allow clinical retention of implants in bone tissue. The biological activity of titanium and its oxides provokes the bone tissue to build up a hard-tissue barrier around the implant and thereby a retention of the prosthetic structure. This bone reaction might be considered as a mild but chronic form of bone defense against a foreign body. The same metal can, however, produce some nondesirable immunological effects in susceptible individuals. Titanium corrodes in the body, especially in presence of fluoride, which is often the case in the oral cavity [86, 87]. Thus, bioavailability of titanium corrosion products may be another basis for immunological reactions. The complement activation, production of inflammatory peptides, production of IL-1 caused by titanium implants was shown by Perala et al already 1991 [88]. Nakashima et al. [89] showed that macrophages could stimulate production of TNF-alpha and IL-6 after exposure to titanium particles. Another possible process is a chronic activation of the immune system, which by a massive cytokine release can lead to a large spectrum of so called psychosomatic or, more adequately, neuropsychiatric symptoms [90, 91]. Stejskal et al. [14] showed memory cell stimulation by titanium in approximately 10% of Swedish patients with ill health associated with dental metals, indicating type IV hypersensitivity reaction.

Especially chronic fatigue, which is seen as one of the dominant symptoms in the present patient group, can easily be associated with immune activation and/or chronic generalized inflammation [92]. The chronic exposure to dental metals in sensitized individuals can be one factor triggering a dysregulation of the hypothalamic-pituitary-adrenal axis by means of chronic inflammation with cascades of symptoms as a result.

All individuals with similar exposure to metals do not experience the same symptoms. The majority of the population is not taken ill at all. A difficulty in the explanation is that there are no typical or specific symptoms, which can be attributed to a specific metal with a high degree of certainty. Symptoms listed in the questionnaire may each of them be provoked by other causes. A typical characteristic in the symptomatology of the present patient group is the multitude of symptoms, their chronic persistence and lack of other reasonable causes. This is well in accordance with chronic toxicity and/or chronic immune dysregulation.

Low-dose exposure to metals can have dramatic effects in a small part of the population. The individual sensitivity may be the reason for this behavior. The individual sensitivity, together with immunological dose-response relations for many substances, including metals, might then be responsible for the resulting ill health. Individual sensitivity to nickel is well recognized and accepted [93]. We do not have any reason to believe that other metals will behave biologically in a completely different way. It is additionally important to have in mind that there is quite a large difference between dose-response relations in toxicology and dose-response relations in immunology.

Metals may exert adverse effects on biological systems in several ways. A basic idea behind the molecular toxicity of, for example, mercury is the affinity for sulfur-containing groups in proteins. By binding to groups such as thiols, the structure of a protein may be changed in a way that makes function altered or impossible. Another possibility is through generation of extremely reactive radicals that can cause oxidative stress. Oxidative stress is widely used, however, rarely defined. The original definition was "a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage" [94]. There

is a multitude of agents causing oxidative stress among them are metals like mercury.

There is a set of defense systems against the reactive oxygen species (ROS) produced during the metabolism of dioxygen in which cytochrome c oxidase catalyses the reduction of O_2 to H_2O. However, the scavenging enzymes are vulnerable to attack from metals like mercury. Such an exposure will impair the function and an increased leakage of ROS has to be expected. In a similar fashion other important biological processes may be interfered with by metal exposure. For example, the biosynthesis of heme is dependent on several enzyme systems susceptible to metal attack. A consequence of an attack on these enzyme systems is a decreased pool of heme. A great number of enzymes is dependent on heme as the prosthetic group. Metal-induced oxidative stress can thus be partially responsible for the toxic effects [95].

It is not difficult to imagine that even minute amounts of metals may be detrimental to a variety of processes in cells thereby impairing their functions. Thus treatment with antioxidants in cases where adverse effects of metal exposure has to be expected is well grounded. There are several critical requirements for a molecule that should be fulfilled in order to create a perfect and effective antioxidant that include but are not limited to: (1) effective radical scavenging, (2) low reactivity of antioxidant radicals towards vital intracellular components and (3) low level of one-electron enzymatic metabolism of antioxidants [96]. The antioxidants included in the treatment of the patients in the present study are well characterized and found to be effective [94, 97].

Many of the symptoms asked about in the questionnaires in the above-cited studies and in the present study were the same as reported at chronic mercury poisoning in mirror platers in the 19th century [98]. This fact together with the clinical experience during the development of diagnostic procedures and treatment protocols indicating subgroups among the patients with different crucial points in their symptom flora did not allow for formulation of an a priori hypothesis about which of the symptoms should be expected to improve on treatment. With the toxic properties of mercury and other metal components of dental restorative materials it is but natural to expect a multitude of symptoms from different organs in the body.

Designs of studies of the effects of amalgam removal were often retrospective. Only a few have been prospective. The retrospective design was adopted for the present study, although there are several known limitations with this design. Clearly defined control groups are scarce in such studies. When controlled studies cannot be performed, a design with the patient or the object as its own control in a before-after situation can be adopted. Such a design is uncontrolled but nevertheless it is a good design annealing the problems of matching controls and makes genetics redundant. The present study comprised a set of consecutive patients. They were consequently not randomized to the study. However, it has recently been concluded that there is a good correlation between the summary odds ratios of randomized and non-randomized studies [99].

Olsson and Lindh [22] used the same questionnaire as Klock et al. [16] and the results were in reasonable agreement. In the study by Olsson and Lindh [22], patients were stratified by the time after completion of amalgam replacement in three groups: 0–3 y, 3–5 y and 5–10 y. Patients who did not begin or did not complete treatment served as a contrast group. Nonparametric ANOVA showed significant differences for all symptoms except “heart troubles” and “trouble with stomach and intestines”. Multiple comparisons showed that the only differing group was the contrast group. Time after replacement did not influence the estimation of improvement. This indeed contradicts the explanation of improvement by placebo effects or the origin to be somatization.

The present study included the symptoms of Klock et al. [16] and Olsson and Lindh [22] in the questionnaire with the objective of making comparisons possible. Table 8 shows a comparison between these studies and the present as far as possible. The results of the Olsson and Lindh [22] study is better for eight symptoms, about equal for three symptoms and worse for one symptom. Comparison with the study of Klock et al. [16] shows better results for two symptoms, equal for one and worse for two. Reasons for these differences are difficult to pinpoint. However, a main difference is that in the studies of Olsson and Lindh [22] and Klock et al. [16], the patients were self-referred in a high degree to dentists and may represent a group with less serious problems. Prevalences were higher of nine out of 12 comparable symptoms in the present study than in Olsson and Lindh [22]. This fact partly supports the hypothesis that patients in the present study had more serious problems. A difference between the study by Klock et al. [16] and most other studies of the current problem is that the distribution of men and women was about the same in the former. Usually, there is a dominance of women. This situation is intriguing because gender effects in toxicological studies have often been neglected [100]. Both biological and non-biological factors may affect the exposure to as well as kinetics and toxicity of metals. There are some contradictions between studies of gender effects. In female mice, higher tissue retention of mercury was observed. However, no effects on the incidence of autoimmunity were demonstrated [101]. On the other hand, Barregård et al. [102] observed three times higher mercury concentration in kidney biopsies from women than from men.

Furthermore, an improvement score was constructed taking into consideration both the change of symptom severity from before to after treatment as well as the estimation of symptom change. Although 60+69=129 patients (27.9%) reported no change of quality of life or a deterioration, there were no negative values in the improvement score. For ten symptoms, these groups reported an improvement score of 0. Although there seems to be on the average no dete-

rioration of symptoms in all groups, there is a clear difference between the positive group and the two other groups. In addition, the estimations of the global variable quality of life certainly create a borderline between groups.

The most frequent symptoms before RID in the positive group were "pain or discomfort in the muscles", "chronic fatigue", "dejection or depression", "fatigue after physical exertion" and "impairment of concentration" in descending order. The effect of RID was most apparent for "chronic fatigue" and "dejection or depression", which after RID were no longer among the five most common symptoms. It is favorable for the RID that two of the most frequent symptoms were substantially decreased in frequency.

Regarding severity in the positive group, the five most frequent symptoms before RID were rather similarly improved with high scores for "chronic fatigue". Interestingly, there was a significantly greater improvement of "blisters and wounds in the mouth" pointing to a direct association to the dental materials.

The improvement score in the positive group also showed that "blisters and wounds in the mouth" was the one with highest scores. The next best improvement was "often infections", which indicates an immunological affection that had been alleviated. "Chronic fatigue" was number four, again pointing to effective treatment of a disabling symptom.

One of the most important findings in this study based on the grouping by quality of life is that it was possible to discriminate between the positive group and the other groups already on information about the symptom status before treatment using a χ^2 test with partitioning. This means that it would be possible to find the group of patients for which there already exists a beneficial treatment. If laboratory tests confirm this discrimination, it would be of great importance in the primary care.

Amalgam-associated ill health has by several groups suggested being due to somatization [7, 103–106]. Arguments used are the lack of correlation between the number of amalgam fillings or the number of amalgam surfaces and adverse health effects. Bailer et al. [107] argue that amalgam-sensitive patients on the average have more medically unexplained symptoms than non-amalgam-sensitive patients and this would be a proof of somatization. However, if the amalgam sensitivity is based on genetics, dose as estimated by number of fillings or surfaces would not have any reasonable meaning. There are indications both from research on experimental animals [82] and estimations for humans [11] that genetics is an important explanatory factor. Another foundation would be the fact that in the present study not all patients were improved in their quality of life. There seems to be no logical explanation for the behavior of the negative and zero groups. Why should their problems of somatization not be alleviated by the treatment?

Somatization has been used, since the beginning of 20th century, as an alternative explanation of symptoms and symptom combinations, for which no physiological explanation could be found with current methods. There are, however, risks with such a wide concept since it has to be continually revised to keep pace with increasing medical knowledge. Patients suffering from borreliosis were transferred from psychiatric clinics to infectious clinics in the 1980s when the causing agent was discovered [108]. Yet another problem was gastric ulcers, the pathogenesis of which was disclosed when the Helicobacter pylori was discovered [109]. Somatization is thus not clearly defined and has unfortunately been used as a medical "slop-pail" rather than a definite differential diagnosis. There are, however, various opinions about somatization. Some groups advocate its importance in being predictor for chronic fatigue [110]. Others, though, report that patients with burning mouth syndrome, as a group, did not report significant psychological distress [111].

When measurements of symptom changes in a group of patients are based on subjective reports in a questionnaire, the question of possible placebo effects will be raised. The placebo effect is, though frequently referred to in the last decades, poorly understood and first of all unsatisfactorily defined. In most descriptions, the placebo effect is mostly considered to be a purely psychological phenomenon resulting in behavioral and emotional changes in the patient. In some theories the placebo includes even some secondary effects like blood pressure changes, increased production of endorphins, hormone balance changes and others physiological processes of non-specific character.

However, the overall opinion seems to express few characteristics intimately associated with placebo effects. The process inducing the placebo is a suggestive impulse that can be of direct or indirect type and of primary or secondary type. The magnitude of response to such suggestive impulses depends on the subject's ability to internalize the suggestion as a temporary cognitive re-structuring. This means that the ability to respond on placebo stimulation should be unevenly distributed in the population. Both placebo reactors and placebo non-reactors have been suggested to represent different personality variables. Further it is generally acknowledged that placebo effects are only of temporary character and not long lasting or self-regenerated. Placebo is generally considered to have a fast, almost immediate effect. The placebo effect is as well considered to abate if the original suggestive stimulus is not repeated. It is not clear where the definitive borders of time-inhibition of the placebo effects are, but a general opinion is that placebo effects last only for a relatively short period such as hours and days, possibly weeks and a few months.

Measurement of the placebo effects is, however, quite impossible in many clinical studies, even studies of pharmacological effects, due to the previously mentioned poor or even non-existing definition of the placebo. That is why, the whole reported positive improvement of the measured variable in a control group is usually considered to be a placebo effect. This meth-

odological impurity, however, neglects other possible physiological variables. These may be fluctuation of symptoms, spontaneous improvement and scaling bias in the measurement of subjective outcomes. In many cases other possible influences are not described, such as beneficial effects of additional treatment or improved life style provided during the actual study. Recently, a more critical view on earlier placebo studies has appeared [112] especially pointing out weaknesses in the original publication from 1955 "The powerful placebo" by Beecher, the conclusions of which still are widely accepted [113].

The health changes between before and after RID in the present patient group are associated with several important characteristics that contradict the presence of a significant placebo effect. The patients have been chronically ill during many years without measurable relief despite numerous treatment efforts from many specialist physicians. This shows at least that these patients did not have the personality type of strong placebo reactors. It is unlikely that they could suddenly, after our treatment, change their reaction pattern to be well responding to placebo stimuli.

Many of the patients experienced an unexpected worsening of their symptoms during the dental treatment phase, which can be associated with the increased exposure to offending substances rather than to a lack of placebo effect. The incidence of patients with no health improvement or even deteriorated health (zero and negative groups) is equally contradicting a strong placebo influence. This contradiction is further supported by the fact that these two sub-groups expressed as high satisfaction with the care at the department as the group with improved health (positive group).

Patients have reported no immediate positive effect on symptoms during the treatment. Most of positive health changes have often appeared after considerable time (normally several months and up to one year) following finished RID. The health changes also seem to be long lasting or even permanent. In fact, in patients of the positive group the health improvement seems to continue even years after the last contact with our medical-dental team. This is not compatible with placebo hypotheses but well compatible with the fact that the patients were liberated from substances causing their health problems. The subjective reports from the patients can be supported by results of accompanying positive changes in a number of biomarkers in different laboratory tests, part of which is discussed here.

Our conclusion is that although the placebo effects are not possible to estimate or directly measure, it is far from likely that the positive health changes in majority of our patients can be attributed to the placebo phenomenon. This is corroborated by the study of Jones [114] arriving at the conclusion that the placebo effect is not supported as an exclusive explanation for positive health outcomes in patients with ill health associated with dental amalgam.

The plasma concentrations of mercury before and after treatment display conspicuous differences between the groups. Results from the positive group support that the improvement of symptoms and quality of life most probably was due to the treatment effects. The after values are in good agreement with concentrations found in umbilical cord blood in mothers with no amalgam fillings [115]. A low concentration before and a small change in the after concentration in the negative group points to other problems. Thus, their current health problems are probably not primarily related to metal exposure but due to other possible intolerances.

Results from a small subgroup ($n=25$) of patients investigated by nuclear microscopy of blood cells [116] show striking similarities with results from nuclear microscopy of patients with inflammatory connective-tissue disease [117, 118]. This is yet another support for the influence on the immune system. Removal of amalgam resulted in substantial improvement of the health and a normalization of nuclear microscopy results [116]. These results are in accord with results from another subgroup ($n=111$) comparing before and after MELISA® tests [14]. There was a marked decrease in lymphocyte reactivity to inorganic mercury as well as to other metals present in dental alloys.

In conclusion, the presented data from the questionnaire and available laboratory findings support the idea that the majority of the patient group suffered from adverse effects of exposure to metals from dental restorative materials. Support is also offered from the fact that other known causes of this kind of ill health had been thoroughly excluded in the primary and specialist care before the patients were referred to the department. Although it was shown to be possible to identify the positive group using the response profile concerning symptoms before treatment, further research is warranted as to the possibilities to develop clinically significant and available laboratory tests to identify the group, for which there is a reasonable hope of successful treatment with current knowledge.

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REFERENCES

1 Westcott A. Report of the Onondaga Medical Society on "Mineral paste". Am J Dent Sci 1844; **4**:137–40.
2 Tuthill JY. Mercurial neurosis resulting from amalgam fillings. The Brooklyn Medical Journal 1899; **12**:725–42.
3 Stock A. Die Gefährlichkeit des Quecksilberdampfes. Z Angew Chemie 1926; **39**:461–6.
4 Stock A. Die Gefährlichkeit des Quecksilberdampfes und der Amalgame. Med Klin 1926; **22**:1209–12 & 1250–2.
5 Stock A. Die chronische Quecksilber- und Amalgamvergiftung. Zahnärtzl Rundsch 1939; **48**:371–7 & 403–7.
6 Bangsi D, Ghadirian P, Ducic S, Morisset R, Ciccocioppo S, McMullen E, Krewski D. Dental amalgam and multiple sclerosis: a case-control study in Montreal, Canada. Int J Epidemiol 1998; **27**:667–71
7 Ahlqwist M, Bengtsson C, Furnäs B, Hollender L, Lapidus L. Number of amalgam tooth fillings in relation to subjectively experienced symptoms in a study of Swedish women. Commun Dent Oral Epidemiol 1988; **16**:227–31.
8 Richardson GM. Assessment of Mercury Exposure and Risks from Dental Amalgam. Final Report. Ottawa: Medical Devices Bureau, Health Canada; 1995.
9 Richardson GM. A Monte-Carlo assessment of mercury exposure and risks from dental amalgams. Human Ecol Risk Assessment 1996; **2**:709–61.
10 Richardson GM. Mercury exposure from dental amalgams: re-evaluation of the Richardson model, standardization by body surface area, and consideration of recent occupational studies. In: Novakova V, editor. Amalgam and health – new perspectives. Report 99:1. Tierp: Swedish Council for Planning and Coordination of Research; 1999. p. 384–418.
11 Berlin M. Mercury in dental fillings: a literature and knowledge survey. In: Novakova V, editor. Amalgam and health – new perspectives. Report 99:1. Tierp: Swedish Council for Planning and Coordination of Research; 1999. p. 369–83.
12 Alroth-Westerlund B, Carlmark B, Grönquist S-O, Johansson E, Lindh U, Theorell H, de Vahl K. Altered distribution patterns of macro- and trace elements in human tissues of patients with decreased levels of blood selenium. Nutr Res 1985; Suppl **I**:442–50.
13 Lindh U, Carlmark B, Grönquist S-O, Lindvall A. Metal exposure from amalgam alters the distribution of trace elements in blood cells and plasma. Clin Chem Lab Med 2001; **39**:134–42.
14 Stejskal VDM, Danersund A, Lindvall A, Hudecek R, Nordman V, Yaqob A, Mayer W, Bieger W, Lindh U. Metal-specific lymphocytes: biomarkers of sensitivity in man. Neuroendocrinol Lett 1999; **20**:289–98.
15 Fleischmann P. Zur Frage der Gefährlichkeit kleinster Quecksilbermengen. Dtsch Med Wschr 1928; **8**:304–7.
16 Klock B, Blomgren J, Ripa U, Andrup B. Effect of amalgam on patients suspected of amalgam poisoning (in Swedish). Tandläkartidningen 1989; **81**:1297–302.
17 Siblerud RL. Health effects after dental amalgam removal. J Orthomol Med 1990; **5**:95–106.
18 Zamm AV. Removal of dental mercury: often an effective treatment for the very sensitive patient. J Orthomol Med 1990; **5**:138–42.
19 Lindqvist B, Mörnstad H. Effects of removing amalgam fillings from patients with diseases affecting the immune system. Med Sci Res 1996; **24**:355–6.
20 Lichtenberg H. Elimination of symptoms by removal of dental amalgam from mercury poisoned patients, as compared with a control group of average patients. J Orthomol Med 1993; **8**:145–8.
21 Godfrey M, Campbell N. Confirmation of mercury retention and toxicity using 2,3-dimercapto-1-propane-sulphonic acid sodium salt (DMPS). J Adv Med 1994; **7**:19–30.
22 Olsson G, Lindh U. Veränderungen des allgemeinen Gesundheitszustandes nach Amalgamentfernung. Ganzh Zahnmed 1997; **2**:22–8.
23 Huggins HA, Levy TE. Cerebrospinal fluid protein changes in multiple sclerosis after dental amalgam removal. Altern Med Rev 1998; **3**:295–300.
24 Kremers L, Halbach S, Willruth H, Mehl A, Weltzl G, Wack FX, et al. Effect of rubber dam on mercury exposure during amalgam removal. Eur J Oral Sci 1999; **107**:202–7.
25 Henriksson E, Mattsson U, Håkansson J. Healing of lichenoid reactions following removal of amalgam. A clinical follow-up. J Clin Periodontol 1995; **22**:287–94.
26 Begerow J, Zander D, Freier I, Dunemann L. Long-term mercury excretion in urine after removal of amalgam fillings. Int Arch Occup Environ Health 1994; **66**:209–12.
27 Ostman PO, Anneroth G, Skoglund A. Amalgam-associated oral lichenoid reactions. Clinical and histological changes after removal of amalgam fillings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996; **81**:459–65.
28 Ibbotson SH, Speight EL, Macleod RI, Smart ER, Lawrence CM. The relevance and effect of amalgam replacement in subjects with oral lichenoid reactions. Br J Dermatol 1996; **134**:420–3.
29 Berglund A, Molin M. Mercury levels in plasma and urine after removal of all amalgam restorations: the effect of using rubber dams. Dent Mater 1997; **13**:297–304.
30 Sandborgh-Englund G, Elinder CG, Langworth S, Schütz A, Ekstrand J. Mercury in biological fluids after amalgam removal. J Dent Res 1998; **77**:615–24.
31 Engel P. Observations on health before and after amalgam removal (in German). Schweiz Monatsschr Zahnmed 1998; **108**:811–3.
32 Molin M, Bergman B, Marklund SL, Schütz A, Skerfving S. Mercury, selenium, and glutathione peroxidase before and after amalgam removal in man. Acta Odontol Scand 1990; **48**:189–202.
33 Halbach S, Kremers L, Willruth H, Mehl A, Weltzl G, Wack FX, et al. Systemic transfer of mercury from amalgam fillings before and after cessation of emission. Environ Res Sect A 1998; **77**:115–23.
34 Björkman L, Sandborgh-Englund G, Ekstrand J. Mercury in saliva and feces after removal of amalgam fillings. Toxicol Appl Pharmacol 1997; **144**:156–62.
35 Melchart D, Wuhr E, Weidenhammer W, Kremers L. A multicenter survey of amalgam fillings and subjective complaints in non-selected patients in the dental practice. Eur J Oral Sci 1998; **106**:770–7.
36 Goldberg RL, Kaplan SR, Fuller GC. Effect of heavy metals on human rheumatoid synovial cell proliferation and collagen synthesis. Biochem Pharmacol 1983; **32**:2763–6.
37 Ganther HE. Modification of methylmercury toxicity and metabolism by selenium and vitamin E: possible mechanisms. Environ Health Perspect 1978; **25**:71–6.
38 Queiro ML, Pena SC, Salles TS, de Capitani EM, Saad ST. Abnormal antioxidant system in erythrocytes of mercury exposed workers. Human and Exp Toxicol 1998; **17**:225–30.
39 Zalups RK, Lash LH. Interaction between glutathione and mercury in the kidney. In: Chang LW, editor. Toxicology of metals. Boca Raton: CRC Press 1996; p. 145–63.

40 Miller OM, Lund BO, Woods JS. Reactivity of Hg(II) with superoxide: evidence for the catalytic dismutation of superoxide by Hg(II). J Biochem Toxcol 1991; **6**:293–8.
41 Tiffany-Castiglione E, Qian Y. Astroglia as metal depots: molecular mechanism for metal accumulation, storage and release. Neurotoxicol 2001; **22**:577–92.
42 Oram PD, Fang X, Fernando Q, Letkeman P, Letkeman D. The formation constant of mercury(II)-glutathione complexes. Chem Res Toxicol 1996; **9**:709–12.
43 Martin RB. Bioinorganic chemistry of metal ion toxicity. In: Sigel H, editor. Metal ions in biological systems. Concepts in metal ion toxicity. New York: Dekker 1986; **20**:21–66.
44 Vimercati L, Santarelli L, Pesola G, Drago I, Lasorsa G, Valentino M, et al. Monocyte-macrophage system and polymorphonuclear leukocytes in workers exposed to low levels of metallic mercury. Sci Total Envion 2001; **270**:157–63.
45 Vimy MJ, Lorscheider FL. Intra-oral air mercury released from dental amalgam. J Dent Res 1985; **64**:1069–71.
46 Worth RG, Esper RM, Warra NS, Kindzelskii AL, Rosenspire AJ, Todd RF, et al. Mercury inhibition of neutrophil activity: evidence of aberrant cellular signalling and incoherent cellular metabolism. Scand J Immunol 2001; **53**:49–55.
47 Bucio L, García C, Souza V, Hernández E, González C, Betancourt M, et al. Uptake, cellular distribution and DNA damage produced by mercuric chloride in a human fetal hepatic cell line. Mutat Res 1999; **423**:65–72.
48 Fonfría E, Rodriguez-Farré E, Suñol C. Mercury interaction with the $GABA_A$ receptor modulates benzodiazepine binding site in primary cultures of mouse cerebellar granule cells. Neuropharmacol 2001; **41**:819–33.
49 Weinberg JM, Hardin PG, Humes HD. Mitochondrial bioenergetics during the initiation of mercuric chloride-induced renal injury. I. Direct effects of in vitro mercuric chloride on renal mitochondrial function. J Biol Chem 1982; **257**:60–7.
50 Weinberg JM, Hardin PG, Humes HD. Mitochondrial bioenergetics during the initiation of mercuric chloride-induced renal injury. II. Functional alterations of renal cortical mitochondria isolated after mercuric chloride treatment. J Biol Chem 1982; **257**:68–74.
51 Lund B-O, Miller DM, Woods JS. Studies on Hg(II)-induced H_2O_2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem Pharmacol 1993; **45**:2017–24.
52 Chevez E, Hougin JA. Mitochondrial calcium release as induced by Hg^{2+}. J Biol Chem 1988; **263**:3582–7.
53 Brawer JR, McCarthy GF, Gornitsky M, Frankel D, Mehindate K, Schipper HM. Mercuric chloride induces a stress response in cultured astrocytes characterized by mitochondrial uptake of iron. Neurotoxicol 1998; **16**:767–76.
54 Rosenspire AJ, Bodepudi S, Mathews M, McCabe MJ, Jr. Low levels of ionic mercury modulate protein tyrosine phosphorylation in lymphocytes. Int J Immunopharmacol 1998; **20**:697–707.
55 Mattingly RR, Felczak A, Chen C-C, McCabe MJ, Jr, Rosenspire AJ. Low concentrations of inorganic mercury inhibit Ras activation during T cell receptor-mediated signal transduction. Toxicol Appl Pharmacol 2001; **176**:162–8.
56 McCabe MJ, Santini RP, Rosenspire AJ. Low and non-toxic levels of ionic mercury interefere with the regulation of cell growth in the WEHI-231 B-cell lymphoma. Scand J Immunol 1999; **50**:233–41.
57 Shenker BJ, Guo TL, Shapiro IM. Mercury-induced apoptosis in human lymphoid cells: evidence that the apoptotic pathway is mercurial species dependent. Environ Res Sect A 2000; **84**:89–99.
58 Wataha JC, Lockwood PE, Schedle A, Noda M. Ag, Cu, Hg and Ni ions alter the metabolism of human monocytes during extended low-dose exposure. J Oral Rehab 2002; **29**:133–9.
59 Oshiro S, Nozawa K, Hori M, Zhang C, Hashimoto Y, Kitajima S, Kawamura K-I. Modulation of iron regulatory protein-1 by various metals. Biochem Biophys Res Commun 2002; **290**:213–8.
60 Hisatome I, Kurata Y, Sasaki N, Morisaki T, Morisaki H, Tanaka Y, et al. Block of sodium channels by divalent mercury: role of specific cysteinyl residues in the P-loop region. Biophys J 2000; **79**:1336–45.
61 Hultman P, Johansson U, Turley SJ, Lindh U, Enestrom S. Adverse immunological effects and autoimmunity induced by dental amalgam and alloy in mice. FASEB J 1994; **8**:1183–90.
62 Sweet LI, Zeliko JT. Toxicology and immunotoxicology of mercury: a comparative review in fish and humans. J Toxicol Environ Health B Crit Rev 2001; **4**:161–205.
63 Schuppe H-C, Rönnau AC, von Schmiedeberg S, Ruzicka T, Gleichmann E, Griem P. Immunomodulation by heavy metal compounds. Clin Dermatol 1998; **16**:149–57.
64 Heo Y, Lee WT, Lawrence DA. In vivo the environmental pollutants lead and mercury causes induction of oligoclonal T cell responses skewed toward type-2 reactions. Cell Immunol 1997; **179**:185–95.
65 Hultman P, Turley SJ, Enestrom S, Lindh U, Pollard KM. Murine genotype influences the specificity, magnitude and persistence of murine mercury-inducec autoimmunity. J Autoimmun 1996; **9**:139–49.
66 Bigazzi PE. Metals and kidney autoimmunity. Environ Health Perspect 1999; **107** (suppl 5):753–65.
67 Fournie GJ, Mas M, Cautain B, Savignac M, Subra JF, Pelletier L, et al. Induction of autoimmunity through bystander effects. Lessons from immunological disorders induced by heavy metals. J Autoimmun 2001; **16**:319–26.
68 Pollard KM, Pearson DL, Hultman P, Deane TN, Lindh U, Kono DH. Xenobiotic acceleration of idiopathic systemic autoimmunity in lupus-prone bsxb mice. Environ Health Perspect 2001; **109**:27–33.
69 Roether S, Rabbani H, Mellstedt H, Abedi-Valugerdi M. Spontaneous downregulation of antibody/autoantibody synthesis in susceptible mice upon chronic exposure to mercuric chloride is not owing to a general immunosuppression. Scand J Immunol 2002; **55**:493–501.
70 Gorell JM, Rybicki BA, Cole Johnson C, Peterson EL. Occupational metal exposure and the risk of Parkinson's disease. Neuroepidemiology 1999; **18**:303–8.
71 Uversky VN, Li J, Fink AL. Metal-triggered structural transformations, aggregation, and fibrillation of human α-synuclein. A possible molecular link between Parkinson's disease and heavy metal exposure. J Biol Chem 2001; **276**:44284–96.
72 Ely JTA. Mercury induced Alzheimer's disease: accelerating incidence? Bull Environ Contam Toxicol 2001; **67**:800–6.
73 Underwood EJ, Mertz W. Introduction. In: Mertz W, editor. Trace elements in human and animal nutrition, 5th ed, vol 1. San Diego: Academic Press, Inc; 1987. p. 1–19.
74 Goyer RA. Toxic and essential metal interactions. Annu Rev Nutr 1997; **17**:37–50.
75 Danning CL, Boumpas DT. Commonly used disease-modifying antirheumatic drugs in the treatment of inflammatory arthritis: an update on mechanisms of action. Clin Exp Rheumatol 1998; **16**:595–604.

76 Zietman AL, Shipley WU, Kaufman DS. The combination of cis-platin based chemotherapy and radiation in the treatment of muscle-invading transitional cell cancer of the bladder. Int J Radiat Oncol Biol Phys 1993; **27**:161–70.
77 Klaassen CD, editor. Casarett and Doull's Toxicology: The Basic Science of Poisons. 5th ed. New York: McGraw-Hill; 1996.
78 Wang S, Shi X. Molecular mechanisms of metal toxicity and carcinogenesis. Mol Cell Biochem 2001; **222**:3–9.
79 Clemens S. Molecular mechanisms of plant metal tolerance and homeostasis. Planta 2001; **212**:475–86.
80 Ragsdale SW. Nickel biochemistry. Curr Opin Chem Biol 1998; **3**:188–99.
81 Mattila L, Kilpeläinen M, Terho EO, Koskenvuo M, Helenius H, Kalimo K. Prevalence of nickel allergy among Finnish university students in 1995. Contact Derm 2001; **44**:218–23.
82 Hultman P, Lindh U, Hörsted-Bindslev P. Activation of the immune system and systemic immune-complex deposits in brown Norway rats with dental amalgam restorations. J Dent Res 1998; **77**:1415–25.
83 Sterzl I, Procházková J, Hrda P, Bártová J, Matucha P, Stejskal VDM. Mercury and nickel allergy: risk factors in fatigue and autoimmunity. Neuroendocrinol Lett 1999; **20**:221–8.
84 Ahlgren C, Ahnlinde I, Björkner B, Bruze M, Liedholm R, Möller H, et al. Contact allergy to gold is correlated to dental gold. Acta Derm Venereol 2002; **82**:41–4.
85 Ahnlinde I, Björkner B, Bruze M, Möller H. Exposure to metallic gold in patients with contact allergy to gold sodium thiosulfate. Contact Dermatitis 2000; **43**:344–50.
86 Strietzel R, Hösch A, Kalbfleisch H, Buch D. In vitro corrosion of titanium. Biomat 1998; **19**:1495–9.
87 Reclaru L, Meyer JM. Effects of fluorides on titanium ond other dental alloys in dentistry. Biomat 1998; **19**:85–92.
88 Perala D, Chapman R, Gefland J. Complement activation by dental implants. Int J Oral Maxollofac Implants 1991; **6**:136–41.
89 Nakashima Y, Sun DH, Trindade MC, Maloney WJ, Goodman SB, Schurman DJ, et al. Signaling pathways for tumor necrosis factor-alpha and interleukin-6 expression in human macrophages exposed to titanium-alloy particulate debris in vitro. J Bone Joint Surg Am 1999; **81**:603–15.
90 McDonald EM, Mann AH, Thomas HC. Interferons as mediators of psychiatric morbidity. Lancet 1978; **Nov 21**, 1175–8.
91 Hickie I, Lloyd A. Are cytokines associated with neuropsychiatric syndrome in humans? Int J Immunopharm 1995; **8**:677–83.
92 Buchwald D, Wener MH, Pearlman T, Kith P. Markers of inflammation and immune activation in chronic fatigue and chronic fatigue syndrome. J Reumatol 1997; **24**:372–6.
93 Hindsen M, Bruze M, Christensen OB. Individual variation in nickel patch test reactivity. Am J Contact Dermatit 1999; **10**:62–7.
94 Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 3ed. Oxford: Oxford University Press; 1999.
95 Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative stress. Curr Top Med Chem 2001; 1:**5**29–39.
96 Kagan VE, Kisin ER, Kawai K, Serinkan BF, Osipov AN, Serbinova EA, et al. Toward mechanism-based antioxidant intervention. Lessons from natural antioxidants. Ann NY Acad Sci 2002; **959**:188–98.
97 Cornelli U, Terranova R, Luca S, Cornelli M, Alberti A. Bioavailability and antioxidant activity of some food supplements in men and women using the D-Roms test as a marker of oxidative stress. J Nutr 2001; **131**:3208–11.
98 Kussmaul A. Untersuchungen über den constitutionellen Mercurialismus und sein Verhältnis zur constitutionelles Syphilis. Würzburg: Stahel'schen Buch- und Kunsthandlung; 1861.
99 Ioannidis JP, Haidich A-B, Pappa M, Patazis N, Kokori SI, Tektonidou MG, et al. Comparison of evidence of treatment effects in randomized and nonrandomized studies. JAMA 2001; **286**:821–30.
100 Vahter M, Berglund M, Åkesson A, Lidén C. Metals and women's health. Environ Res Sect A 2002; **88**:145–55.
101 Hultman P, Nielsen JB. The effect of dose, gender, and non H-2 genes in murine mercury-induced autoimmunity. J Autoimmun 2001; **17**:27–37.
102 Barregård L, Svalander C, Schütz A, Westberg G, Sällsten G, Blohme I, et al. Cadmium, mercury, and lead in kidney cortex of the general Swedish population: A study of biopsies from living kidney donors. Environ Health Perspect 1999; **107**:867–71.
103 Björkman L, Pedersen NL, Lichtenstein P. Physical and mental health related to dental amalgam fillings in Swedish twins. Commun Dent Oral Epidemiol 1996; **24**:260–7.
104 Bratel J, Haraldson T, Ottosson JO. Potential side effect of dental amalgam restorations (II). No relation between mercury levels in the body and mental disorders. Eur J Oral Sci 1997; **105**:244–50.
105 Langworth S, Sällsten G, Barregård L, Cynkier L, Lindh ML, Söderman E. Exposure to mercury vapor and impact on health in the dental profession in Sweden. J Dent Res 1997; **76**:1397–404.
106 Malt UF, Nedrum P, Oppedal B, Gunderson R, Holte M, Löne J. Physical and mental problems attributed to dental amalgam fillings: a descriptive study of 99 self-referred patients compared with 272 controls. Psychosom Med 1997; **59**:32–41.
107 Bailer J, Rist F, Rudolf A, Staehle HJ, Eickholz P, Triebig G, et al. Adverse health effects related to mercury exposure from dental amalgam fillings: toxicological or psychological causes? Psychol Med 2001; **31**:255–63.
108 Lang J. Catching the bug: How scientists found the cause of Lyme disease. Conn Med 1989; **53**:357–64.
109 Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R. Helicobacter pylori virulence and genetic geography. Science 1999; **284**:1328–33.
110 Addington AM, Gallo JJ, Ford DE, Eaton WW. Epidemiology of unexplained fatigue and major depression in the community: the Baltimore ECA follow-up, 1981–1994. Psychol Med 2001; **31**:1037–44.
111 Carlson CR, Miller CS, Reid KI. Psychosocial profiles of patients with burning mouth syndrome. J Orofac Pain 2000; **14**:59–64.
112 Hróbjartsson A, Gøtzsche PC. Is placebo powerless? An analysis of clinical trials comparing placebo with no treatment. N Engl J Med 2001; **345**:1594–602.
113 Beecher HK. The powerful placebo. JAMA 1955; **159**: 1602–6.
114 Jones L. Dental amalgam and health experience: exploring health outcomes and issues for people medically diagnosed with mercury poisoning. Bull New Zealand Psychol Soc 1999; **97**:29–33.
115 Vahter M, Åkesson A, Lind B, Björs U, Schütz A, Berglund M. Longtudinal study of methylmercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood. Environ Res Sect A 2000; **84**:186–94.

116 Lindh U, Frisk P, Nyström J, Danersund A, Hudecek R, Lindvall A, et al. Nuclear microscopy in biomedical analysis with special emphasis on clinical metal biology. Nucl Instr and Meth 1997; **B130**:406–18.
117 Hällgren R, Svensson K, Johansson E, Lindh U. Elevated granulocyte strontium in inflammatory arthritides is related to the inflammatory activity. J Lab Clin Med 1984; **104**:893–900.
118 Hällgren R, Svensson K, Johansson E, Lindh U. Abnormal calcium and magnesium stores in erythrocytes and granulocytes from patients with inflammatory connective tissue diseases. Relationship to inflammatory activity and effect of cortico-steriod therapy. Arthritis Rheum 1985; **28**:169–73.

APPENDIX

THE FIRST CATEGORY in the questionnaire was comprised of 30 questions about symptoms. These questions were also subdivided into:

A. Do you currently have troubles with the following symptoms? (Yes–No)
B. How serious are/were your troubles? (1–5; 1: no troubles, 5: serious troubles)
   This question was asked pertinent to three stages:
   Before, during and after RID
C. Has the symptom changed? (1–7; 1: much better, 4: unchanged, 7: much worse)

Symptoms included in the first category

1. Chronic or periodic fatigue with duration of at least six months and that cannot be cured with rest in bed and that have inflicted a reduction of daily activities with at least 50%.
2. Feeling cold, shivering or fever?
3. Sore throat, other throat problems?
4. Aching lymphatic glands on the neck or in armpits?
5. Muscle discomfort in the whole body?
6. Pain or discomfort in the muscles?
7. Abnormal fatigue after physical exertion?
8. Headache?
9. Troubles in the joints?
10. Light sensitivity?
11. Have you experienced any temporary impairment of vision?
12. Do you have an impaired memory (forgetfulness)?
13. Are you overly irritated?
14. Do you experience bewilderment?
15. Do you have difficulties to think?
16. Impairment of concentration?
17. A feeling of dejection or depression from time to time?
18. Impairment of sleep?
19. Dizziness or unsteadiness?
20. Buzzing in your ears (tinnitus)?
21. Discomfort in hands/feet?
22. Tremor or spasms in muscle/muscles?
23. Aching shoulder/shoulders?
24. Troubles with the heart?
25. Troubles with stomach/intestines?
26. Troubles with the urinary bladder/urinary production?
27. Eczema, blisters or other skin troubles?
28. Often infections?
29. Blisters, wounds or other discomfort in the mouth?
30. Aching teeth, jaws or face?

APPENDIX *(cont.)*

THE SECOND CATEGORY comprised questions about quality of life and health. The questions concerned the gross health – physically and mentally – as well as the quality of life during different periods; before, during and after RID. The patients were asked to assess their gross health and quality of life on a scale from 1 (very bad) to 7 (very good).

Questions included in this category:

Physically

31. How did you feel physically before the RID in question?
32. How did you feel physically during the RID?
33. How have you been physically during the last year?
34. How have you been physically during the last week?

Mentally

35. How did you feel mentally before the RID in question?
36. How did you feel mentally during the RID?
37. How have you been mentally during the last year?
38. How have you been mentally during the last week?

Quality of life

39. How would you like to describe your total quality of life before the RID in question?
40. How would you like to describe your total quality of life during the RID in question?
41. How would you like to describe your total quality of life during the last year?
42. How would you like to describe your total quality of life during the last week?

THE THIRD CATEGORY comprised questions about the care and activities at the former Department of clinical metal biology. The patients were asked to assess their opinions on a scale from 1 (very bad) to 7 (very good).

Questions included in this category:

43. What is your opinion about the waiting time before you got an appointment at the Department of clinical metal biology?
44. What is your opinion about the treatment and investigation at as well as the interaction with the Department of clinical metal biology