

MORTALITY

Mortality excess in individuals with elevated IgA anti-transglutaminase antibodies: The KORA/MONICA Augsburg cohort study 1989–1998

Marie-Hélène Metzger¹, Margit Heier¹, Markku Mäki², Enzo Bravi³, Andrea Schneider¹, Hannelore Löwel¹, Thomas Illig¹, Detlef Schuppan^{4,5} & Heinz-Erich Wichmann¹

¹GSF – National Research Center for Environment and Health, Institute of Epidemiology, Neuherberg, Germany; ²Medical School – Pediatric Research Centre, University of Tampere, Tampere, Finland; ³Eurospital, Trieste, Italy; ⁴Med. Clinic I, University of Erlangen–Nürnberg, Erlangen, Germany; ⁵Division of Gastroenterology and Hepatology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MS, USA

Accepted in revised form 2 March 2006

Abstract. *Objectives:* Immunoglobulin A (IgA) autoantibodies to tissue transglutaminase (tTG) are commonly used for screening and diagnosing of celiac disease. We examined the hypothesis that elevated IgA anti-tTG antibodies were associated with higher all-cause mortality risk. *Methods:* The cohort, 2333 men and 2300 women, was based on the follow-up of participants of a representative population-based survey in Southern Germany (KORA/MONICA Augsburg project) conducted in 1989–1990. The endpoint for the vital status with cause of death was the year 1998. The sera drawn at baseline and stored at -80°C , were recently screened with an IgA enzyme-linked immunosorbent assay (ELISA) using human recombinant tTG. Age-standardized mortality rates and age-adjusted hazard ratios were calculated. *Results:* From the

4633 sera analyzed, 63 had an IgA anti-tTG concentration ≥ 7 AU/ml. Of these 63 individuals, 15 died between 1989 and 1998. The age-adjusted hazard ratio (HR_a) of all-cause mortality was 1.86 (95% CI: 1.01–3.41) and 3.92 (95% CI: 1.44–10.71) for men and women, respectively. The excess of cancer mortality was even higher with an HR_a of 2.47 (95% CI: 0.89–6.83) in men and of 6.65 (95% CI: 2.04–21.63) in women. *Conclusions:* Individuals with elevated IgA anti-tTG antibodies had a highly increased mortality risk, particularly due to cancer. New studies are necessary to clarify if this increased risk is due to undiagnosed celiac disease or/and if this elevated IgA anti-tTG antibodies level is a marker of serious diseases like cancer, chronic liver disease or end-stage heart failure.

Key words: Celiac disease, Cohort studies, Mortality, Neoplasms, Tissue transglutaminase

Introduction

Tissue transglutaminase (tTG) is an intracellular enzyme, which is highly expressed in fibroblasts and endothelial cells and is released from the cells into the extracellular matrix after tissue injury [1, 2]. Tissue transglutaminase plays a key role as autoantigen in celiac disease, a disorder of the small intestine with autoimmune characteristics [3]. Despite the high positive predictive value of elevated Immunoglobulin A (IgA) anti-tTG-levels for celiac disease [4–6], the autoantibodies were also found in a high proportion of patients suffering from end stage heart failure [7], liver disease [8] and other autoimmune diseases [9, 10]. Clinical manifestations of celiac disease range from asymptomatic to severe malabsorption, but in any case autoantibodies of tTG–IgA are typically found in these patients. Therefore tTG–IgA is an important serologic mar-

ker, used to screen patients with suspected celiac disease [4, 11].

In the context of an epidemiological project, aiming to evaluate the prevalence of celiac disease in Europe, sera drawn of a representative sample of the Southern German population in 1989–1990 and stored for about 10 years were screened by human recombinant anti-tTG–IgA-test.

Our aim was to analyze whether elevated IgA anti-tTG antibodies were associated with higher all-cause mortality risk and cancer mortality.

Methods

Study population

The study is part of KORA (Cooperative Health Research in the Region of Augsburg), which is the

continuation of the earlier MONICA (Multinational MONitoring of trends and determinants in Cardio-vascular disease) project. Sera were drawn from the participants of the second population-based survey of the MONICA Augsburg Project [12], conducted in 1989–1990. The study area was located in Southern Germany and comprised the city of Augsburg and two surrounding counties. A sex–age stratified two-stage cluster sample was drawn from the population registers of the study area. The design has been described in detail elsewhere [13]. A random sample of 6637 persons of German nationality was drawn from the population of 349,050 inhabitants aged 25–74 years. The subjects were invited to one of the 19 examination centers distributed over the whole study area. The response was 76.9%, i.e. 4940 of the 6420 eligible people (eligible people are $n=6637$ minus those who had died or migrated after initial sampling or errors in the population register). Participants who refused to give blood or participants without enough serum for analysis were excluded ($n=307$, i.e. 6.2%), leaving 4633 participants for the analysis presented here.

This study was approved by the Bavarian Ethics Committee.

Data collection at baseline examination

Baseline data on sociodemographic characteristics, cigarette smoking habits, alcohol consumption, medical symptoms and medication use were gathered by trained nurses by the means of a standardized face-to-face interview. Thereafter, the participants underwent a medical examination including collection of a non-fasting blood sample. Since 1989–1990, the sera were stored in a deep freezer at -80°C . For the statistical analysis, age was treated as continuous parameter. The level of education was categorized into ≤ 10 years of education vs. >10 years of education, alcohol consumption into ≤ 60 g alcohol intake per day vs. >60 g for men and into ≤ 30 g alcohol intake per day vs. >30 g for women. Cigarette smoking was dichotomized as current regular smokers vs. never, former or occasional (<1 cigarette/day) smokers, the self-rated health status as very good or good vs. fair or poor.

Laboratory methods

Recently, an IgA enzyme-linked immunosorbent assay (ELISA) that uses human recombinant tTG has been developed by Eurospital, Eu-tTG® umana. In the context of an epidemiological project involving four European centers (Finland, Italy, Ireland, Germany) and aiming to evaluate the prevalence of celiac disease in Europe, the sera of the KORA/MONICA Augsburg project were sent packed in dry ice to the company. All sera were assayed under

standardized conditions during the year 2001. The antibody concentrations were expressed in arbitrary units (AU), i.e. as percentages of the positive reference serum. The cut-off value was fixed at 7 AU/ml. The sensitivity (Se) was determined by testing 82 biopsy proven celiacs (Se = 96.3%) and the specificity (Sp) by testing 97 controls (Sp = 96.9%). These serum samples were provided by three of the four centers involved in the European project (i.e. Finland, Italy and Ireland). Sera with antibody concentrations ≥ 7 AU/ml were labeled as IgA anti-tTG positive.

Follow-up and end points

In 1998, vital status was collected for all participants through the population registries inside and outside the study area. Of the 4633 participants with available serum at baseline, 323 had died. For 13 persons, the vital status could not be assessed because the participant had moved to a foreign country or to an unknown location. Death certificates were transmitted by the local health departments and were coded for the underlying and accompanying causes of death by a single trained person using the 9th revision of the International Classification of Diseases (ICD-9). For one deceased person, the cause of death was not available.

The median follow-up period was 7.95 years with a minimum of 11 days and a maximum of 8.85 years.

Statistical analysis

Some baseline characteristics were compared between individuals with a tTG antibody concentration ≥ 7 AU/ml and those with a lower concentration. The corresponding age-adjusted odds-ratios were derived from logistic regression models. Mortality rates per 1000 person-years were calculated by age and sex. Age-standardized mortality rates per 1000 person-years were calculated by direct standardization using the population of the Federal Republic of Germany in 1989 as the standard population. Cox proportional hazards models were used to evaluate the independent effect of elevated IgA anti-tTG antibodies (IgA anti-tTG ≥ 7 AU/ml) on the all-cause mortality and the cancer mortality. Results are presented as age-adjusted hazard ratios (HR_a) with their 95% confidence intervals. The assumption of proportionality of hazards was checked by fitting the model, and then plotting the log $[-\log(\text{survival})]$ curves to evaluate the parallelism. No severe deviations from parallelism were shown. A p -value of 0.05 is stated as statistically significant. All analyses were performed by using SAS software, LOGISTIC and PHREG procedures (Version 8.2 PC; SAS Institute Inc, Cary, NC).