Flexible Dose-Response Estimation of the Effect of Gamma-Glutamyltransferase on Risk of Cancer Incidence in a Large Prospective Male Cohort

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Background I

- In clinical practice, measurement of **Gamma-glutamyltransferase (GGT)** is commonly used as an **indicator for hepatobiliary disease** and as a **biological marker of excessive alcohol intake**[1–5]

- Several epidemiologic studies have sparked further interest in **elevated GGT as an independent predictor for morbidity and mortality from causes other than liver disease:**
  - GGT independently associated with **cardiovascular disease**[6–11]
  - GGT correlated with most **cardiovascular risk factors**[12–15]
  - Association of GGT with **chronic kidney disease**[16]
  - Independent role of GGT for **premature death from all causes**[9,12,17]

Background II

- **Association of GGT with cancer remains largely unexplored to date!**

- Several **experimental models** elucidated the **ability of cellular GGT to modulate crucial redox-sensitive functions**, such as antioxidant/antitoxic defenses and cellular proliferative/apoptotic balance, and its role in tumor progression, invasion, and drug resistance has been proposed[18–21]

- A potentially interesting, though speculative interpretation subsumes **GGT as a biomarker of exposure to certain cancer-causing xenobiotics**, including persistent organic pollutants (POPs)[22, 23]
  - (e.g. lead, cadmium, dioxins or organochlorine pesticides)

Study Aims

- To flexibly **investigate the dose-response association of serum GGT with risk of overall and site-specific cancer incidence** in a large, prospective, population-based cohort from Austria

Methods I – Study Population

- **Vorarlberg Health Monitoring & Promotion Program**[25–27]
  - **One of the world's largest ongoing population-based risk factor surveillance programs!**
  - Conducted by the **Agency for Social and Preventive Medicine** in Vorarlberg (the westernmost province of Austria) since 1985
  - **All adults of the regions** are invited to participate by a combination of different measures, such as written invitations, television, radio, and newspaper reports.
  - **Active, longitudinal follow-up with repeated routine health examinations** of participants is performed through a recall system of written biennial re-invitation letters.

Methods II – Data Collection & Cancer Ascertainment

- Measurements of **height, weight, smoking status** and **serum GGT** levels are routinely obtained for each study participant.
- **Occupational status** (blue collar, white collar or self-employed) was determined by the insurance number of participants and used as a surrogate measure of socioeconomic status.
- Cancers were identified by the **Vorarlberg cancer registry**, which has been accepted for IARC publication since 1993[28] and has high completeness of recording[29]
- Cohort data linked with the **Vorarlberg Death Index** to identify deaths and to calculate person years at risk.

Methods III – Statistical Analyses

- Association of **baseline log-GGT levels** with overall and site-specific cancer incidence using **Cox proportional hazards regression**, adjusted for age, BMI, smoking status, occupational status, and year of enrollment into cohort

- **Flexible dose-response estimation** of the association of **average GGT levels** (arithmetic means of GGT concentrations during individual follow-up of each study participant) using **non-parametric b-spline expansions of GGT**
  - Calculations based on an algorithm described by Cao et al.[32]
  - Model selection based on **Akaike Information Criterion (AIC)**[34]
    - Penalized likelihood taking into account the number of variables estimated in the model, compromising between a good fit and a parsimonious model.

Methods IV – Regression Spline Analyses

- A GGT concentration of 25 units/L, as the midpoint of the laboratory range of normal GGT values (0-50 U/L in men), was used as **reference value** with a relative risk equalling 1.00.

- All models were adjusted for baseline values of age, BMI, smoking status, occupational status, and year of enrollment into the cohort.

- 95% confidence intervals were computed based on asymptotic normality of the point-wise risk estimates.[32]

Results I

Table 1. Characteristics of study population

All male VHM&PP participants, 1985-2003 (*n*)	80,224
Participants with complete and valid data on GGT (*n*)	79,363
Eligible participants for analyses (*n*)	79,279*
Total number of visits (*n*)	270,281
No. visits, mean ± SD (range)	3.5 ± 2.8 (1-19)
Age (y), mean ± SD (range)	41.6 ± 14.6 (18-96)
BMI (kg/m^2), mean ± SD (median)	25.3 ± 3.6 (24.9)
GGT (units/L), median (range)	28.6 (2.0-798.0)
Current cigarette smoking (%)	30.0
Former cigarette smoking (%)	8.5
Occupational status	
Blue collar (%)	37.6
White collar (%)	51.9
Self-employed (%)	10.5
Follow-up (y), mean ± SD (median)	11.3 ± 5.6 (12.5)
Total person-years at risk	894,231
Incident cancers, *n* (%)	5,305 (6.7)
Age at cancer diagnosis (y), mean ± SD (range)	57.0 ± 12.2 (19-93)

NOTE: GGT values are shown as averages of each participant during individual follow-up and before eventual cancer diagnoses. All other characteristics pertain to baseline values (i.e., measurements at first visit).

*Participants with GGT values >800 units/L or with history of cancer before enrollment were excluded.

Results II

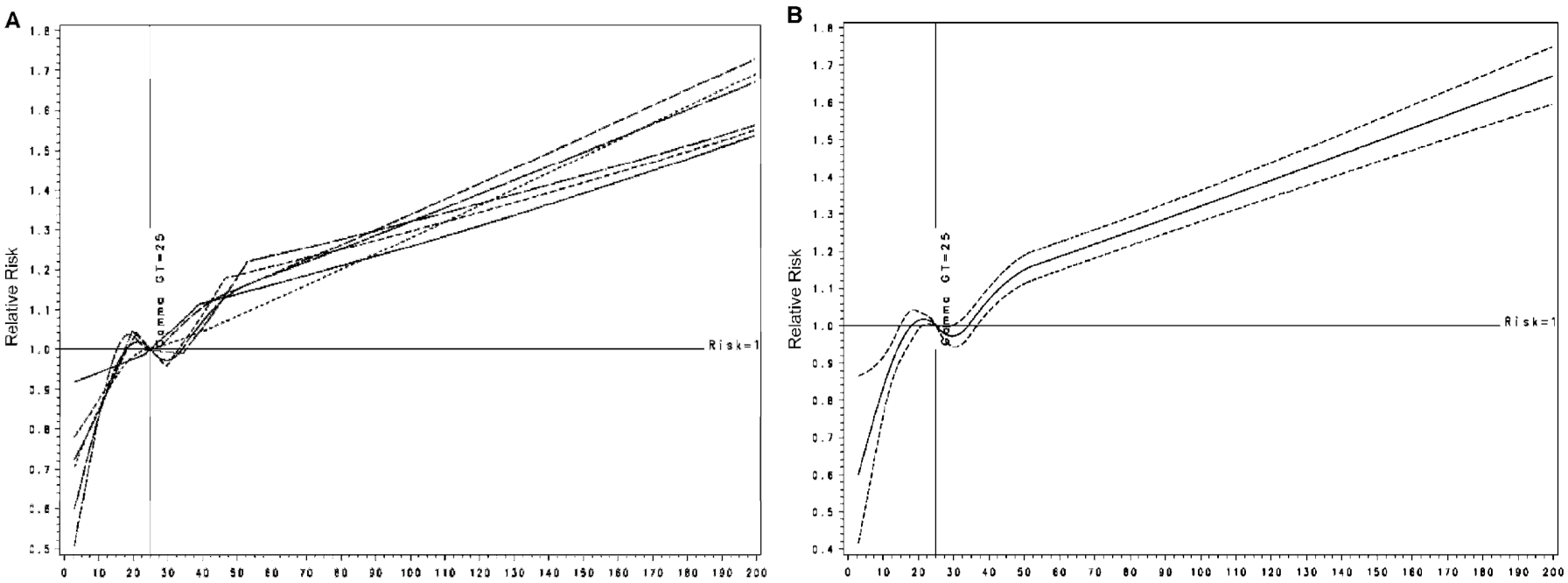
- Elevated GGT statistically significantly increased overall cancer risk, showing a **clear dose-response relationship** (P for GGT log-unit increase <0.0001)

- **Best-fitting Spline Model (2 degrees of freedom, 4 knots at consecutive quintiles of the GGT distribution)**: In comparison with the GGT reference concentration (25 units/L), we found adjusted relative risks (95% CI) equalling

  - **1.19 (1.15-1.22) for 60 units/L**
  - **1.32 (1.28-1.36) for 100 units/L**
  - **1.67 (1.60-1.75) for 200 units/L**
  - **2.30 (2.14-2.47) for 400 units/L**

- In cancer site-specific models, GGT was significantly associated with malignant neoplasms of digestive organs, the respiratory system/intrathoracic organs, and urinary organs (all P<0.0001).

Results III



- **Regression Spline Models:** Relative risk estimates for the association of GGT (0-200 U/L) and overall cancer incidence in 79,279 male Austrian adults

  A) Different linear (df=1) and quadratic (df=2) regression spline models for 2 to 4 knots

  B) AIC-minimizing, best-fitting spline model with df=2, knots=4, and 95%CI (dotted lines)

Results IV

Age of participants significantly modified the association of GGT and overall cancer risk (P for multiplicative interaction <0.001), revealing **markedly stronger associations in participants ages <65 years**.

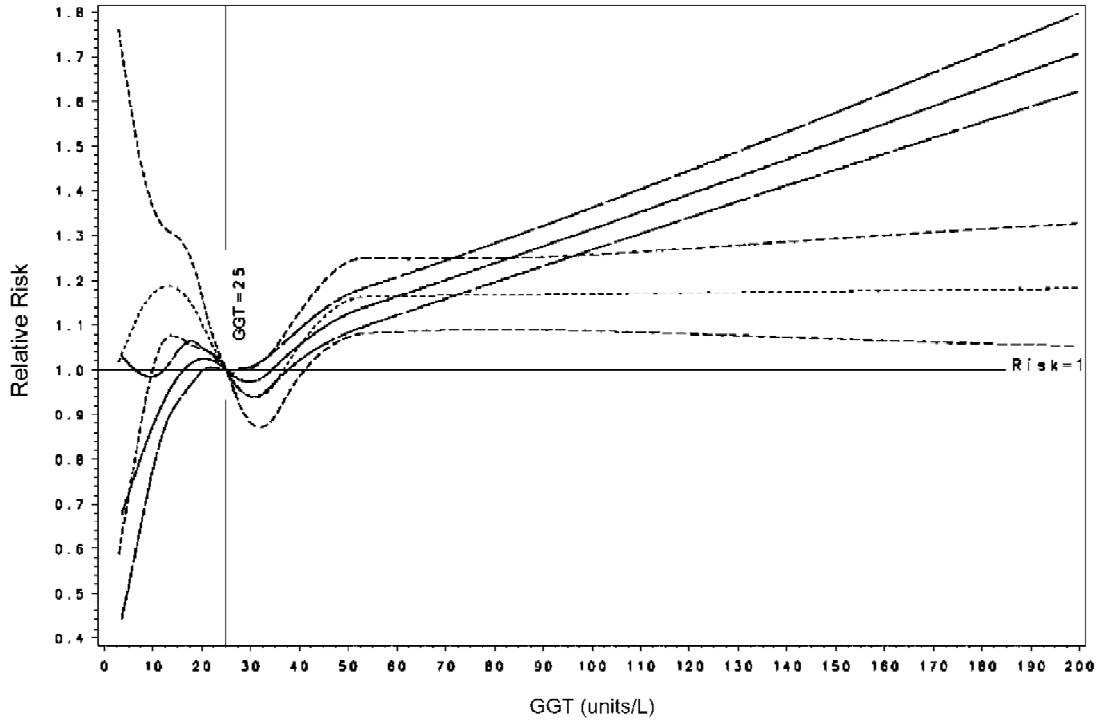


Figure 3. Relative risk estimates with 95% CIs for the association of GGT (range, 0-200 units/L) and overall cancer incidence in participants ages ≤65 y (*n* = 73,278; 3,979 incident cancers; *solid lines*) and participants ages >65 y (*n* = 6,001; 1,326 incident cancers; *dashed lines*). Relative risks were estimated from individual, best-fitting regression spline models. Models are adjusted for age, BMI, smoking status, occupational status, and year of examination. A GGT concentration of 25 units/L was used as reference value with a relative risk of 1.00.

Discussion I

- First epidemiologic investigation to explore the association of GGT with risk of subsequent cancer incidence in men from the general population

- GGT statistically significantly associated with overall cancer incidence and several **life-style triggered malignancies** (digestive organs, the respiratory system/intrathoracic organs, and urinary organs)

- Results largely agree with our recent findings for women in the same cohort!

Discussion II – Potential Limitations of Study

- Information on some risk and confounding factors was not generally available, including physical activity, diet, and alcohol consumption.
  - Only a moderate, age-adjusted correlation of 0.15 ($p<0.05$) of GGT with the average number of alcohol units per week in our cohort.
- Data on drug prescriptions/medication use and prevalent health conditions not routinely collected in our cohort.

Discussion III – Conclusion

- The underlying biological mechanisms causing elevated GGT to increase incidence of cancer overall and for several anatomic sites need further study!

→ **GGT as a new and sensitive marker for a life-style at increased risk for the development of cancer**

Thank you for your attention!

Questions, Comments, etc.: **alexander.strasak@i-med.ac.at**

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Supplemental Table 1. Akaike Information Criterion values for linear and quadratic regression spline models with 2-4 knots for the association of GGT and risk of overall and site-specific cancer incidence, VHM&PP 1985-2003*

Regression Spline Models†		All Cancers	Site-specific cancers						
			Digestive Organs	Respiratory System and intrathoracic organs	Bone, connective tissue, soft tissue and skin	Genital organs	Urinary organs	Nervous system and unspecified sites	Lymphoid, haematopoietic and related tissue
Linear	2 knots	31920.46	12274.80	9824.36	**7392.57**	16393.05	7040.46	4607.89	**6213.04**
	3 knots	31913.93	12272.98	9820.22	7393.48	**16389.60**	**7039.83**	4605.32	6215.02
	4 knots	31913.64	12272.33	9812.98	7394.19	16389.96	7040.52	**4600.94**	6213.67
Quadratic	2 knots	31914.00	**12263.04**	**9808.80**	7392.75	16393.91	7044.03	4603.90	6215.31
	3 knots	31913.65	12265.09	9810.07	7394.80	16396.15	7043.49	4605.20	6214.83
	4 knots	**31913.53**	12266.24	9810.81	7395.99	16392.24	7044.05	4603.69	6216.73

*The optimal number of segments was selected by putting an increasing number of knots at subsequent tertiles (2 knots), quartiles (3 knots) and quintiles (4 knots) of the GGT distribution, and the best-fitting model was defined as the one minimizing the Akaike Information Criterion (AIC), a penalized likelihood that takes into account the number of parameters estimated in the model, compromising between a good fit and a parsimonious model.

†All models are adjusted for age, body-mass index, smoking status, occupational status and year of examination. Bold numbers with smaller AIC values indicate best-fit models.