Non-invasive detection of any-stage cancer using free glycosaminoglycans

Francesco Gatto[1,2], Sinisa Bratulic[1], Ilaria Teresa Rita Cavarretta[3], Massimo Alfano[3], Francesca Maccari[4], Fabio Galeotti[4], Nicola Volpi[4], Per-Henrik Edqvist[5],Max Levin[6,7,8], Jan Nyman[7,8], Bengt Bergman[9], Andrea Salonia[10,11], Sven Lundstam[7,12], Ulrika Stierner[8], Henrik Kjölhede[12,13], Jens Nielsen[1], U-CAN consortium

Affiliations:
1) Department of Biology and Biological Engineering, Chalmers University of Technology, 412 96 Göteborg, Sweden; 2) Elypta AB; 3) Division of Experimental Oncology/Unit of Urology, URI, IRCCS Ospedale San Raffaele, Milan, 20132, Italy; 4) Department of Life Sciences, University of Modena and Reggio Emilia, 411 25 Modena, Italy; 5) Dept. Immunology, Genetics & Pathology, Uppsala University, 751 85 Uppsala, Sweden; 6) Department of Molecular and Clinical Medicine/Wallenberg Laboratory, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; 7) Department of Oncology, Institute of Clinical Sciences, Sahlgrenska Academy at University of Gothenburg, Sweden; 8) Department of Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden; 9) Department of Respiratory Medicine, Institute of Medicine, The Sahlgrenska Academy, University of Gothenburg, 413 45 Göteborg, Sweden; 10) Vita-Salute San Raffaele University , Milan, Italy, 11) Division of Experimental Oncology/Unit of Urology; URI; IRCCS Ospedale San Raffaele, Milan, Italy; 12) Departments of Urology, Sahlgrenska Academy, University of Gothenburg, 41345 Göteborg, Sweden; 13) Department of Urology, Sahlgrenska University Hospital, 413 45, Göteborg, Sweden

Background

- Early detection can save cancer patient lives.
- Liquid biopsies promise to enable early detection but:
  - They mostly address genomics biomarkers;
  - They perform poorly in some tumors (genitourinary/brain);
  - They have limited sensitivity to stage I/low-grade disease.
- We previously found that glycosaminoglycan profiles (**GAGomes**) may be potential biomarkers of metabolic reprogramming in cancer – specifically, in renal cell carcinoma (*1-3*).

Objectives

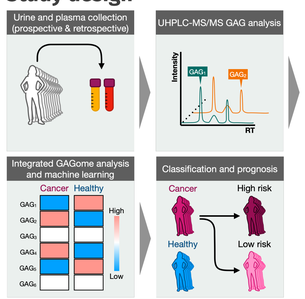
1. Are GAGomes in urine and plasma altered compared to physiological levels across multiple cancer types (besides renal cell carcinoma)?
2. Can we develop models (GAG scores) based on GAGomes that discriminate between any-stage any-type cancer vs. healthy controls?

Study Design and Methods

- Case-control study totaling 1502 samples
  - Cases: Cancer patients of 14 types (N = 553)
  - Controls: Healthy subjects (N = 426)
- Quantification of free GAGomes in plasma (N = 942) and urine (N = 560) using UHPLC-MS/MS kit (4) in a central blinded lab
- Bayesian logistic regression on any-cancer vs healthy to develop 3 GAG score: plasma-only, urine-only, or combined GAG scores (discovery vs validation set 60%:40% samples)
- Performance evaluation in terms of AUC and sensitivity at 98% specificity (any-cancer vs healthy). Subset analysis: stage I/low-grade*
- Bayesian Additive Regression Tree model to predict tissue-of-origin
- Kaplan-Meier survival analysis on overall survival between “low” versus “high” GAG score for each score, with optimal cut-off to dichotomize patients (cancer arm only)

*“Stage I/low grade” included cancers with TNM (8th edition) or FIGO or Ann Arbor stage I or ENETS grade 1 or Gleason grade < 7 or non-grade IV glioma; “Stage IV/high-grade” included cancers with TNM (8th edition) or FIGO or Ann Arbor stage IV or ENETS grade 2 or Gleason grade >= 7 or grade IV glioma; “Stage II” and “Stage III” included cancers with the corresponding stage number in the TNM (8th edition) or FIGO or Ann Arbor systems.

Study design



Patient characteristics

	Overall (N=979)
Age	
Mean (SD)	61.3 (13.5)
Median [Min, Max]	64.0 [21.0, 91.0]
Gender	
Female	499 (51.0%)
Male	480 (49.0%)
Group	
Healthy (H)	426 (43.5%)
Bladder cancer (BCa)	47 (4.8%)
Breast cancer (BC)	28 (2.9%)
Cervical cancer (CST)	28 (2.9%)
Chronic lymphocytic leukaemia (LL)	18 (1.8%)
Colorectal cancer (CRC)	27 (2.8%)
Diffuse Glioma (DG)	40 (4.1%)
Diffuse large B-cell lymphoma (NHL)	30 (3.1%)
Endometrial Carcinoma (EC)	30 (3.1%)
Head and Neck Cancer (HN)	17 (1.7%)
Non-small-cell Lung Carcinoma (NSCLC)	83 (8.5%)
Ovarian Carcinoma (OV)	30 (3.1%)
Prostate cancer (PCa)	104 (10.6%)
Renal Cell Carcinoma (RCC)	57 (5.8%)
Small Intestinal Neuroendocrine Tumor (GNET)	14 (1.4%)

Urine and plasma free glycosaminoglycan (GAG) profiles – the GAGomes - aggregated in GAG scores:

- Detected any-stage, any-type cancer with a sensitivity up to 40.5% at 98% specificity
- Predicted tissue-of-origin with 74.3% accuracy
- Independently correlated these with overall survival

Urine and plasma GAG scores were robust and versatile metabolic liquid biomarkers for early multicancer detection - detecting up to 33% stage I/low grade cancers as well as brain and genitourinary tumors historically missed by genomics-based liquid biopsies.

Future directions

- External validation in cohort studies of patient population of interest for early multicancer detection
- Comparison of GAG scores complementarity with genomics-based liquid biopsies in case-control studies.

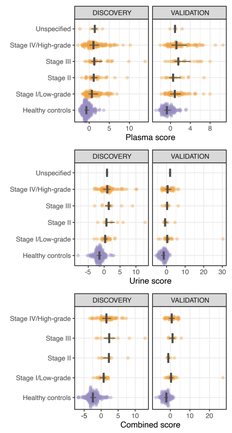
Results

GAG scores performance for any-stage any-cancer vs. healthy controls

Plasma (top), urine (middle), and combined (bottom) pan-cancer GAG scores across different stage/grade groups* and ROC curve in the discovery (60% samples) and validation set (40%).

In the validation set, the sensitivity to any-stage cancer for the plasma, urine, and combined pan-cancer GAG score was 38.6%, 34.6% and 40.5% respectively.

In the subset of stage I/low-grade tumors, the sensitivities were 30.9%, 33.3% and 33.3%, respectively.



PLASMA

Set	AUC (95% CI)	Sensitivity (95% CI) at 98% specificity
Discovery	0.829 (0.796-0.862)	37.2 (31.8-42.8)
Validation	0.838 (0.798-0.877)	34.6 (28.1-41.6)
Stage I	0.804 (0.742-0.866)	30.9 (20.2-43.3)
Stage I-II	0.798 (0.74-0.855)	31.4 (21.8-42.3)
Stage I-III	0.822 (0.772-0.872)	33.3 (24.7-42.9)

URINE

Set	AUC (95% CI)	Sensitivity (95% CI) at 98% specificity
Discovery	0.885 (0.845-0.926)	51.5 (42.7-60.3)
Validation	0.817 (0.759-0.875)	38.6 (28.4-49.6)
Stage I	0.825 (0.721-0.93)	33.3 (15.6-55.3)
Stage I-II	0.807 (0.716-0.898)	29 (14.2-48)
Stage I-III	0.809 (0.728-0.889)	30 (16.6-46.5)

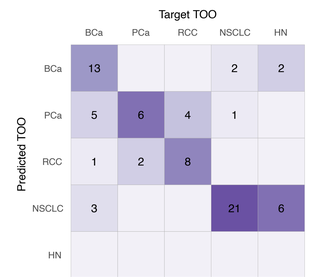
COMBINED

Set	AUC (95% CI)	Sensitivity (95% CI) at 98% specificity
Discovery	0.931 (0.901-0.961)	47.3 (37.7-57)
Validation	0.864 (0.813-0.916)	40.5 (29.3-52.6)
Stage I	0.871 (0.789-0.952)	33.3 (16.6-57)
Stage I-II	0.854 (0.78-0.927)	29.6 (13.8-50.2)
Stage I-III	0.872 (0.811-0.933)	34.3 (19.1-52.2)

GAGome profiles can predict tissue of origin prediction

Tissue of origin (TOO) prediction in the validation set (N = 74, 5 cancer types) using a Bayesian Additive Regression Trees model trained on combined GAGomes in the discovery set (N = 110). The numbers in the boxes represent the number of samples classified as belonging to the predicted TOO.

The balanced accuracy in TOO prediction in the validation set was 74.3% (95% CI = 68.1%-80.3%).

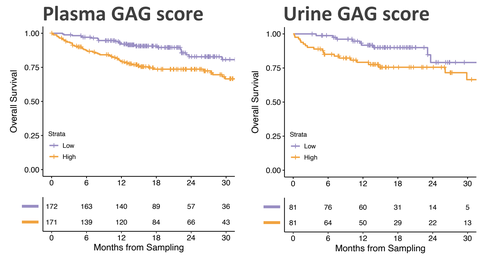


GAG scores correlated with overall survival

Kaplan–Meier curves for overall survival (OS) in the cancer arm stratified into groups of “Low” (purple) vs. “High” (orange) pan cancer GAG score values based on an optimal cut-off.

The hazard ratio (HR) for OS adjusted for age, gender, type and stage IV were:

- Plasma: HR = 1.87 [95% CI = 1.36-2.57], p < 0.001, N = 370, 13 types
- Urine: HR = 2.50 [95% CI = 1.50-4.16], p < 0.001, N = 162, 4 types)



References
1. Gatto et al. (2014): “*Chromosome 3p loss of heterozygosity is associated with a unique metabolic network in clear cell renal carcinoma*”, PNAS
2. Gatto et al. (2016): “*Glycosaminoglycan Profiling in Patients' Plasma and Urine Predicts the Occurrence of Metastatic Clear Cell Renal Cell Carcinoma*”, Cell Reports
3. Gatto et al. (2018): “*Plasma glycosaminoglycans as diagnostic and prognostic biomarkers in surgically treated renal cell carcinoma*”, Eur. Uro. Oncology
4. Tamburro et al. (2021): “*Analytical Performance of a Standardized Kit for Mass Spectrometry-based Measurements of Human Glycosaminoglycans*”, Jounral of Chromatography B