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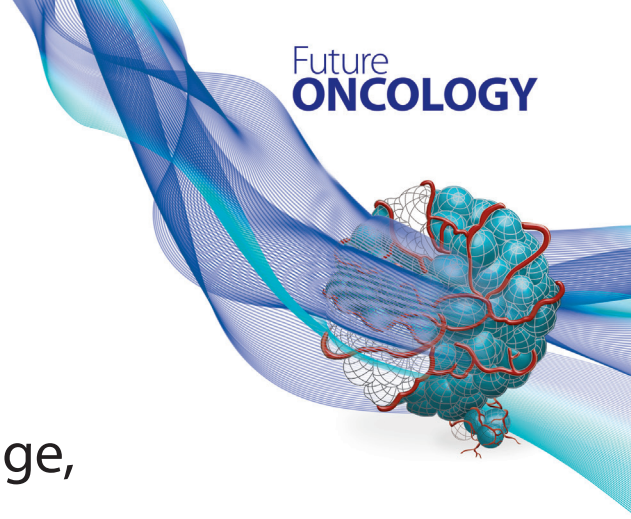

Plasma *N*-acetyl-glucosaminidase in advanced gastro-intestinal adenocarcinoma correlates with age, stage and outcome

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ABSTRACT **Background:** *N*-acetyl-glucosaminidase (NAG) is a potential marker of genotoxicity. We retrospectively analyzed plasma NAG and clinico-pathologic features in advanced gastrointestinal adenocarcinoma patients. **Methods:** Plasma from 118 patients and 51 healthy volunteers was analyzed for associations between NAG levels and age, disease presence, stage, treatment responses and survival. **Results:** Pretreatment NAG correlated with age but was independently increased in metastatic versus locally advanced disease, particularly in gastric/esophageal patients. NAG was also associated with reduced overall survival. In subgroup analysis, increased NAG activity between day 1 and 2 of chemotherapy cycle 1 correlated with treatment response. **Conclusion:** We demonstrated that NAG correlates with gastrointestinal cancer outcomes. Further studies are required to determine if plasma markers of genotoxicity can be useful for disease monitoring.

KEYWORDS

• aging • biomarkers • cancer • fluoropyrimidines • gastrointestinal adenocarcinoma • *N*-acetyl-glucosaminidase • senescence

Background

Biological age is a risk factor in many cancers and the molecular factors underpinning cellular aging including telomere dysfunction and DNA damage responses have been shown to be common features at early stages of carcinogenesis [1]. These molecular events have been investigated in tumor samples by approaches including fluorescence hybridization and immunohistochemistry [2–4] and are intrinsically involved in cell aging and cellular senescence, correlating with tumor initiation of gastrointestinal tumors, such as adenocarcinoma of the stomach and colon [5]. There is considerable current interest in clinical application of markers of cellular senescence in cancer [6,7]. However, the 'senectome' is poorly defined and therefore there is a need to define the most appropriate markers for use in different settings. Furthermore, in clinical practice, there is a need for markers that are easy to measure in patient material and that are accessible to repeat sampling, ideally in blood for treatment monitoring.

Recently, we described the identification of secreted proteins including the Chitinase enzyme *N*-acetyl-glucosaminidase (NAG) that are elevated in aging human fibroblasts preceding the onset of senescence and in response to DNA damage. NAG can be detected in human blood and increases with

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human aging [8]. In this study, we investigated its association with gastrointestinal adenocarcinoma. We measured plasma NAG levels in 118 patients and 51 healthy volunteers to examine associations with objective treatment response to chemotherapy and with overall survival in addition to other clinico-pathologic features.

• Patients & methods

The recruitment criteria and sampling methods used in the current study were previously reported in [9]. The study was approved by the West Glasgow Hospitals Research Ethics Committee, and all patients gave written informed consent before undertaking any study-related procedures. The study was designed to investigate potential biomarkers of response to palliative fluoropyrimidine-based chemotherapy in advanced gastrointestinal cancer patients. Eligible patients were those attending for chemotherapy treatment at a single institution (the Beatson West of Scotland Cancer Center, Glasgow, UK) with histologically or cytologically confirmed, locally advanced or metastatic gastrointestinal adenocarcinoma who were due to start systemic anticancer therapy, with either adenocarcinoma of the colon or rectum receiving fluoropyrimidine-based therapy or adenocarcinoma of the oesophagus or stomach receiving chemotherapy with a regimen containing a fluoropyrimidine and a platinum analogue. Additional inclusion criteria were age ≥18 years, ability to comply with study procedures and life expectancy ≥3 months. Patients who had systemic anticancer therapy or radiotherapy within the previous 6 weeks were excluded, as were women who were pregnant or lactating.

Plasma samples were obtained from 118 patients with gastrointestinal cancer who were treated at the Beatson West of Scotland Cancer Center, and from 51 healthy volunteers (control group). The volunteers in this retrospective analysis were not age-matched. Chemotherapy was administered according to local protocols. 20 ml of blood were collected before starting chemotherapy (baseline) and before administration of each subsequent course of chemotherapy [9]. Disease response was assessed clinically and by computed tomography scans performed before commencing chemotherapy and at regular intervals during treatment (after cycles 3 and 6 for patients with gastric or esophageal cancer and patients with colorectal cancer receiving capecitabine, and after cycles 6 and 12 in patients with colorectal cancer receiving infusional 5-flourouracil-based regimens). Disease response, as defined by the RECIST criteria [10], was determined from the radiology reports for individual patients. Epithelial toxicity experienced by patients during chemotherapy was assessed by review of an individual patient's case records, but detailed toxicity assessments were not prospectively recorded in this study [9].

Enzyme activity of NAG was determined with the Chitinase assay kit on baseline (pretreatment) samples (CS1030; Sigma, MO, USA) according to the manufacturer's instructions and our previous report [8]. 2 µl of plasma was used per reaction. A control sample was included in quadruplicate on each assay plate to control for intra- and inter-plate variance. Plasma levels in patients versus volunteers, in locally advanced versus metastatic disease, and relation between NAG levels and patient response were analyzed by Mann-Whitney U-test and logistic regression. Correlation to age was determined by Spearman rank correlation test. Association between baseline (pretreatment) levels and primary disease site was assessed by Kruskal–Wallis test. To test for a correlation between baseline levels and overall survival, Cox regression analysis was performed, with NAG examined continuously.

Results

• NAG positively correlates with age

We have previously shown that NAG is increased in unaffected elderly individuals by comparison with healthy young individuals [8,11]. To determine whether this association held in the current patient group, we compared baseline levels within the patient group using Spearman's rank correlation test. Patient characteristics are given in **Table 1**. Median age was 68.5 years. As shown in **Figure 1A**, positive correlation was observed ($r = 0.334$). Gender was not associated with baseline levels in the patient group by Mann–Whitney test **(Figure 1B**, NAG: female 29.81 U/ml, male 33.44 U/mLl, $p = 0.151$).

We next analyzed baseline (pretreatment) levels of NAG in plasma samples of the patient group (colorectal, gastric, and esophageal cancer patients) compared with healthy volunteers. **Figure 1C** shows these results. Median activity in the patient group was higher at 32.1 U/ml compared with 23.65 U/ml in the control group ($p < 0.001$, Mann–Whitney test). However, in this retrospective study the volunteer group was not age-matched and was significantly younger ($p < 0.001$) than the patients **(Table 1)**. Results of

Table 1. Patient and volunteer characteristics.

Characteristic	Number
Patients: age (years)	
Median (68.5)	
Range (24–88)	118
Patients: gender	
Female	49
Male	69
Patients: primary tumor site	
Gastroesophageal	58
Colorectal	60
Patients: disease extent	
Locally advanced	24
Metastatic	94
Patients: chemotherapy	
Capecitabine	48
Capecitibine/oxaliplatin	6
FOLFIRI	1
5-flourouracil/folinic acid	5
ECF/ECX	51
Platinum/5-flourouracil	7
Patients: best response	
Partial response	21
Stable disease	49
Disease progression	36
Non-evaluable	12
Healthy volunteers: age (years)	
Median (37.0)	
Range (22–55)	51
Healthy volunteers: gender	
Female	26
Male	25

a logistic regression analysis incorporating age and gender in addition to NAG levels (Table 2) showed that age was the only independent predictor of disease presence among these variables. Similar results were obtained when NAG activities were discretised to above- or below-median groups (Table 2).

Correspondingly, we asked whether age, gender and study status (patient/control) influence the discretized NAG categories (Table 3). In this analysis, both age and gender are significantly associated with NAG activity, with the hazard ratio indicative of higher activities in males. However, study status was not significant. Spearman rank correlation including all individuals (patients and controls) gave a stronger correlation than patients only (Figure 1D, r = 0.527). NAG activity is thus strongly associated with age as previously observed, both in the patient cohort and in the entire group. Hence, age-matching will be required in future studies to address whether NAG is upregulated in patients relative to controls.

• NAG is elevated in metastatic disease in patients with esophago-gastric cancer

We next assessed whether primary cancer site (gastric, colorectal, esophageal) is associated with baseline levels of NAG. No significant difference was detected by Kruskal–Wallis test (Figure 2A, p = 0.672). However, comparing locally advanced (24 patients) versus metastatic disease (94 patients), NAG was elevated in the metastatic group (Figure 2B, median 32.10 U/ml compared with 28.36 U/ml for locally advanced disease, p = 0.043 by Mann–Whitney).

In sub-group analysis of the disease stage according to tumor site, 58 patients had esophago-gastric adenocarcinoma (19 with locally advanced disease and 39 with metastatic disease). NAG was again elevated in the

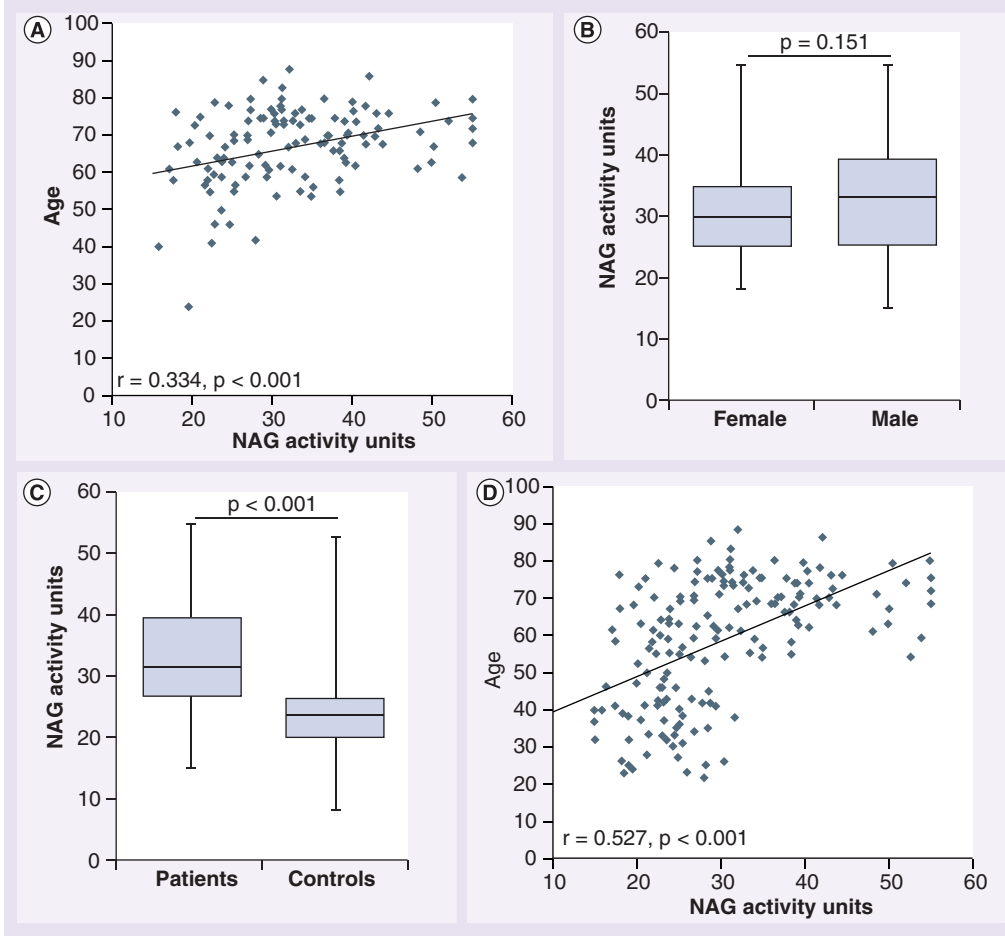

Figure 1. *N*-acetyl-glucosaminidase correlates with age in plasma of gastrointestinal cancer patients and controls. (A) Baseline levels of NAG correlate with age in the patient group. Positive correlation was analyzed by Spearman's rank correlation test. **(B)** No association between patient gender and NAG levels. P-values were calculated by Mann–Whitney test. **(C)** NAG is elevated in plasma of gastro-intestinal cancer patients. Baseline levels of NAG were measured in plasma of 118 gastrointestinal cancer patients and 51 healthy controls. P-values were calculated by Mann–Whitney test. **(D)** Stronger correlation of baseline NAG levels with age after inclusion of the healthy controls. Positive correlation was analyzed by Spearman's rank correlation test.
NAG: *N*-acetyl-glucosaminidase.

Table 2. Logistic regression model of predictors of presence of disease.

Predictors of presence of disease	N	B	SE	p-value	Hazard ratio [Exp(B)]	95% CI for Exp(B)	
						Lower	*Upper*
NAG baseline	169	-0.046 (0.007)	0.049 (0.81)	0.346 (0.993)	0.955 (1.007)	0.868 (0.206)	1.051 (4.924)
Age	169	0.239 (0.218)	0.044 (0.038)	<0.001 (<0.001)	1.270 (1.244)	1.165 (1.156)	1.383 (1.339)
Gender	169	-1.184 (-1.004)	0.716 (0.692)	0.098 (0.147)	0.306 (0.366)	0.075 (0.094)	1.245 (1.423)

118 patients and 51 healthy controls were included in the analysis. Values are results obtained with NAG treated continuously or, in brackets, discretised to above- or below-median.
B: Unstandardized regression coefficients; N: Number of individuals included in the analysis; NAG: *N*-acetyl-glucosaminidase; SE: Standard errors associated with the coefficients.

Table 3. Logistic regression model of predictors of *N*-acetyl-glucosaminidase activity in 118 patients and 51 controls.

Predictors of NAG activity	N	B	SE	p-value	Hazard ratio [Exp(B)]	95.0% CI for Exp(B)	
						Lower	*Upper*
Age	169	0.087	0.021	<0.001	1.090	1.046	1.136
Gender	169	-0.827	0.383	0.031	0.437	0.207	0.926
Patient/control	169	-0.108	0.701	0.878	0.898	0.227	3.546

B: Unstandardized regression coefficients; N: Number of individuals included in the analysis; NAG: *N*-acetyl-glucosaminidase. SE: Standard errors associated with the coefficients.

metastatic group (Figure 2C) with median activity of 33.04 U/ml compared with 25.75 U/ml for locally advanced disease (p = 0.019). Hence, NAG activity associates with more advanced disease in esophago-gastric adenocarcinoma in the current cohort. The remaining 60 patients had colorectal cancer of which only five had locally advanced and 55 had metastatic disease. This number of patients with locally advanced disease is too small to allow an adequately powered comparison. To confirm that NAG association with metastatic disease is independent of other variables, we again performed logistic regression analysis, incorporating primary site, age, gender and NAG baseline activity (Table 4). In this analysis, primary site, NAG baseline and patient age were all statistically significant. However, because the limited number of locally advanced colorectal cancer patients will influence the contribution of primary site in this analysis, we also tested the other variables without including primary site (Table 4, in parentheses). The results were similar for NAG activity in both analyses. However, NAG activity was the only significant predictor variable when primary site was excluded. Hence, pretreatment NAG activity is an age-independent predictor of metastatic disease in the current patient group.

• Dynamic changes in NAG levels on chemotherapy initiation associate with treatment response

To determine whether there is correlation between baseline NAG levels and objective tumor response, response rates were determined both for the entire patient group and for the individual tumor sites (partial response or stable disease versus progressive disease). No significant association between response rate and baseline levels was observed in the whole cohort (PR+SD, n = 70; PD, n = 36; p = 0.219 by Mann–Whitney, Figure 3A). Similarly, no association between baseline levels and response rate was found by analysis of individual tumor sites (Figure 3B). However, in a subgroup of 24 esophago-gastric patients from whom plasma samples had been taken on both

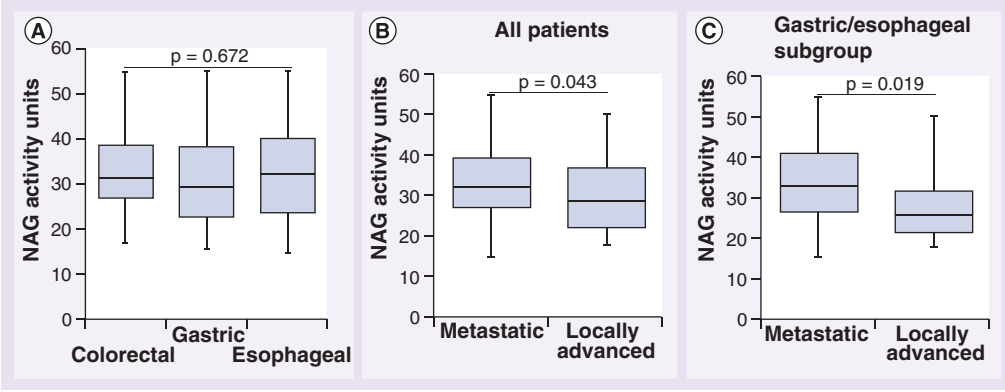

Figure 2. *N*-acetyl-glucosaminidase activity is associated with disease progression in gastric/esophageal cancer patients. (A) No association between baseline plasma levels of NAG and primary tumor site analyzed by Kruskal–Wallis test. **(B)** Elevated baseline plasma NAG levels in metastatic compared with locally advanced disease in the whole patient group, or **(C)** in a subgroup of 58 patients with gastric or esophageal cancers. P-values were calculated by Mann–Whitney test. NAG: *N*-acetyl-glucosaminidase.

Table 4. Logistic regression model of predictors of disease stage.

Predictors of disease stage	N	B	SE	p-value	Hazard ratio [Exp(B)]	95.0% CI for Exp(B)	
						Lower	*Upper*
Primary site	118	-1.967 (n/a)	0.594 (n/a)	<0.001 (n/a)	0.140 (n/a)	0.044 (n/a)	0.448 (n/a)
NAG baseline	118	0.083 (0.076)	0.032 (0.032)	0.010 (0.017)	1.087 (1.080)	1.020 (1.014)	1.158 (1.149)
Age	118	-0.060 (-0.034)	0.029 (0.024)	0.041 (0.170)	0.942 (0.967)	0.890 (0.922)	0.998 (1.014)
Gender	118	0.455 (0.409)	0.533 (0.490)	0.393 (0.403)	1.576 (1.506)	0.555 (0.576)	4.480 (3.936)

Values are results obtained with or without (parentheses) inclusion of primary site in the model.
B: Unstandardized regression coefficients; N: Number of individuals included in the analysis; n/a: Not applicable;
NAG: *N*-acetyl-glucosaminidase; SE: Standard errors associated with the coefficients.

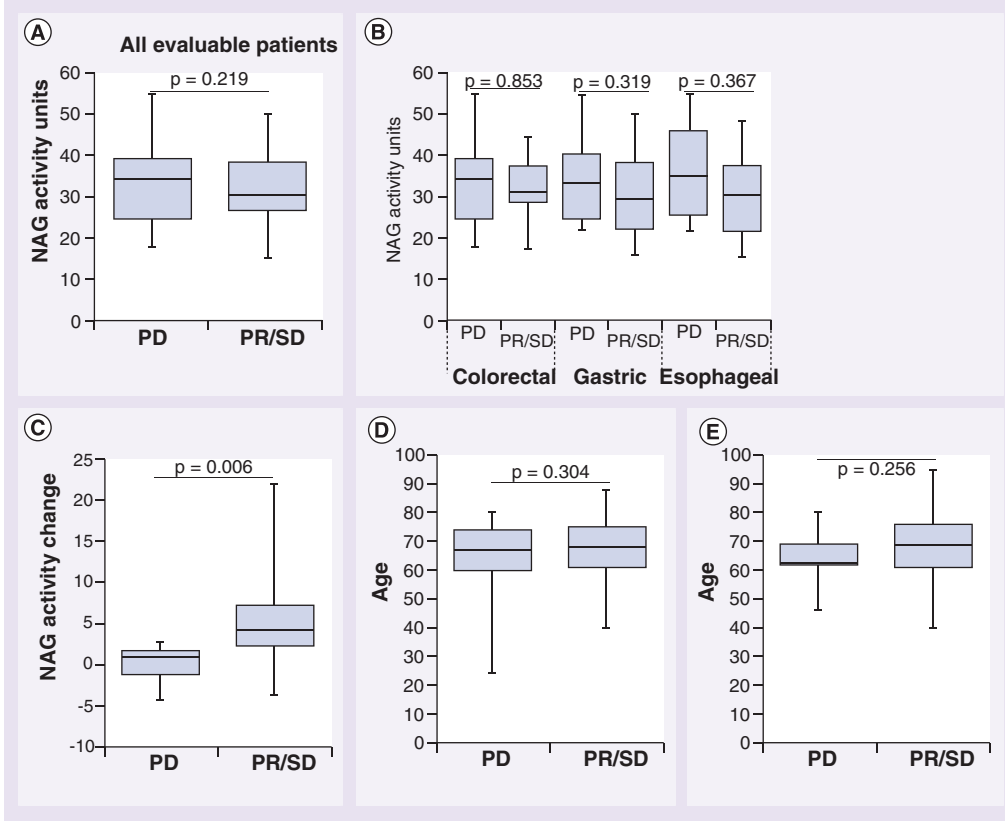

Figure 3. Dynamic changes in *N*-acetyl-glucosaminidase activity in early chemotherapy predict treatment responses. *N*-acetyl-glucosaminidase activity was assessed in the groups partial response plus stable disease versus progressive disease. (A) No association between baseline levels of NAG activity in the whole cohort and treatment responses. P-values were calculated by Mann–Whitney test. **(B)** No association between baseline levels of NAG activity and treatment responses by primary site. P-values were calculated by Mann–Whitney test. **(C)** A subset of 28 patients had plasma samples taken on both treatment days 1 and 2 of chemotherapy cycle 1. Increased NAG activities were detected in the PR/SD group between the treatment days. P-values were calculated by Mann–Whitney test. **(D)** No association between age and treatment response in the whole cohort. P-values were calculated by Mann–Whitney test. **(E)** No association between age and treatment response in the treatment day 1–2 subgroup. P-values were calculated by Mann–Whitney test.
NAG: *N*-acetyl-glucosaminidase; PD: Progressive disease; PR: Partial response; SD: Stable disease.

day 1 and day 2 of treatment cycle 1, we found significant changes in NAG levels between samples, with the magnitude of change (day 2 levels – baseline) significantly greater in the responders (n = 16) than the non-responders (n = 8, **Figure 3C**, p = 0.006, Mann–Whitney test).

In particular, NAG activity is substantially increased on treatment day 2 in the stable disease/responding group, but the levels were comparatively unaffected in patients with progressive disease. No association between age and response was observed by Mann–Whitney analysis, either in the whole cohort (**Figure 3D**, p = 0.304) or in the subgroup sampled on both treatment days (**Figure 3E**, p = 0.256). Furthermore, in a logistic regression analysis incorporating age, sex and disease stage, NAG activity change remained the only significant independent predictor of response to therapy **(Table 5)**. Hence, these results appear age-independent. Therefore, increased NAG activity shortly after initiation of chemotherapy appears to predict treatment response.

• NAG levels significantly correlate with poor overall patient survival

Of 118 patients, 115 were dead and three alive at the time of analysis. In order to test for a correlation between baseline levels and overall survival, Cox regression analysis was performed examining NAG baseline levels, age, gender and disease stage. Baseline NAG levels were the only significant predictor in this analysis (p = 0.033). Hence, baseline NAG levels correlate with overall survival **(Table 6)**. In order to further examine the association between NAG levels and overall survival, the data were split according to quartiles and log rank test was performed on the survival distributions of the upper and lower quartiles (**Figure 4A**, p = 0.045). High pretreatment levels of NAG were associated with poor overall survival in the current cohort. Median survival for the quartile of patients with highest NAG activity (Q4) was 6.2 months compared with 12.3 months for the quartile (Q1) of patients with lowest NAG activity in plasma (**Figure 4B**, p = 0.016 by Mann–Whitney). Spearman rank correlation analysis in all patients also revealed a significant negative correlation between NAG levels and survival time (**Figure 4C**; r = -0.228; p = 0.014). Hence, high plasma NAG levels were associated with lower survival times in these patients.

Discussion

NAG has previously been identified as a secreted marker of DNA damage that is upregulated prior to onset of cellular senescence and that associates with human aging and the evolution of age associated disease, such as Alzheimers' disease [8,11,12]. Here, we report a study of the levels in plasma of patients with gastro-intestinal adenocarcinoma. It could not be determined in the current retrospective study whether NAG is increased in this patient group relative to healthy volunteers because age-matching was not performed. Furthermore, the current study was retrospective and it was unfortunately not possible to identify an age-matched subgroup from the volunteers of sufficient size to permit any robust statistical analysis from the available samples.

However, in line with our previous findings, NAG activity strongly correlated with age both in the patient group and in all individuals. These results provide further evidence that plasma NAG is a marker of human organismal aging. This is the first time that age dependence of NAG activity has been demonstrated within a cancer patient population which is itself an important finding, but the observed age dependence may also be a feature of other candidate markers of cellular senescence. Hence, future prospective studies designed to validate this or other senescence markers should incorporate age-matching not only of controls/patients but also, for example, of treatment arms.

Within the patient group, NAG was found to be elevated in metastatic disease both in the

Table 5. Logistic regression model of predictors of treatment response.

Predictors of treatment response	N	B	SE	p-value	Hazard ratio [Exp(B)]	95.0% CI for Exp(B)	
						Lower	*Upper*
Age	24	0.065	0.060	0.279	1.067	0.949	1.201
NAG change	24	0.504	0.249	0.043	1.656	1.016	2.700
Gender	24	-0.506	1.111	0.649	0.603	0.068	5.326
Disease stage	24	0.968	1.256	0.441	2.634	0.225	30.863

B: Unstandardized regression coefficients; N: Number of individuals included in the analysis; NAG: *N*-acetyl-glucosaminidase; SE: Standard errors associated with the coefficients.

Table 6. Correlation of baseline *N*-acetyl-glucosaminidase levels and overall survival in Cox regression model.

Correlation with overall survival	N	B	SE	p-value	Hazard ratio [Exp(B)]	95.0% CI for Exp(B)	
						Lower	*Upper*
NAG baseline	118	0.024	0.011	0.033	1.024	1.002	1.047
Gender	118	-0.301	0.193	0.119	0.740	0.507	1.081
Disease stage	118	0.315	0.242	0.193	1.370	0.853	2.200
Age	118	-0.009	0.009	0.339	0.991	0.974	1.009

B: Unstandardized regression coefficients; N: Number of individuals included in the analysis; NAG: *N*-acetyl-glucosaminidase; SE: Standard errors associated with the coefficients.

whole patient cohort and in the esophago-gastric subgroup. Levels did not associate with tumor primary site and no clear relation was found between baseline (pretreatment) levels and objective treatment response either in the entire cohort or in tumor-site subgroups. However, in a subset

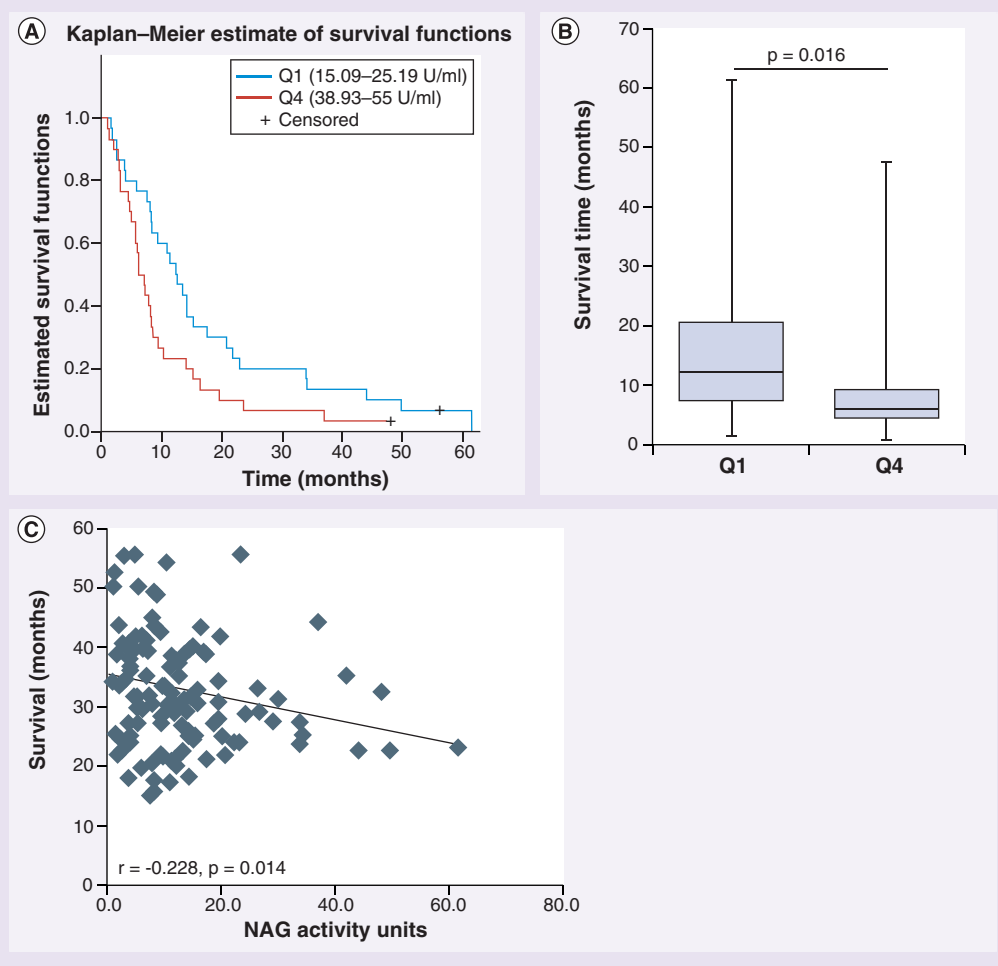

Figure 4. High baseline *N*-acetyl-glucosaminidase activity associates with poor overall survival in advanced gastrointestinal adenocarcinoma. (A) Kaplan–Meier plot of overall survival in the upper (Q4, n = 30) and lower (Q1, n = 30) quartiles of NAG activity demonstrating that high activity corresponds with poor outcome. Results of the Cox regression analysis are given in Table 6. **(B)** Survival time distributions in the Q1 and Q4 groups. P-values were calculated by Mann–Whitney test. **(C)** Inverse correlation between NAG levels and survival time analyzed by Spearman rank correlation. NAG: *N*-acetyl-glucosaminidase.

of patients from whom plasma was collected on both treatment day 1 and day 2, increases in activity following first treatment were associated with tumor response to therapy and NAG change was the only independent predictor in a logistic regression model incorporating age, gender and disease stage. Furthermore, baseline NAG levels were significantly associated with poor outcome in this study (hazard ratio 1.024) in Cox regression analysis incorporating age, gender and disease stage. These results may reflect the higher pretreatment expression observed in metastatic disease.

It will be of considerable interest to investigate dynamics of NAG plasma activity in response to therapy and as a possible predictor of outcome in future prospective studies. It is possible that this increase reflects an acute response to therapy given the relation between this marker and DNA damage responses. High baseline levels in the nonresponders that were unaffected may be indicative of chronic DNA damage signaling in the tumor and resistance to therapy. This study was designed specifically to investigate potential biomarkers of response to palliative fluoropyrimidine-based chemotherapy and this is the first demonstration that cytotoxic therapies can affect plasma NAG in this group. It will therefore be of considerable interest to investigate the potential for radiotherapy and other DNA-damaging chemotherapies to produce similar effects on NAG in future studies. Indeed, one possible application of plasma NAG is as a candidate marker of treatment–induced cellular senescence. Since the current study excluded patients treated with systemic therapy within 6 weeks of enrollment, we believe that potentially confounding effects of prior treatment with other agents, which might affect NAG, should not affect our analysis and interpretation.

Substantial current interest in senescence induction as a potential therapeutic modality in cancer treatment implies a need for markers of senescence responses with utility in the clinical setting [7]. It is widely held that a robust senescence signature will comprise several well-defined and easily assayed markers [6,13–15]. NAG activity may be a candidate since it has been linked with telomere dysfunction and is known to be upregulated in presenescent cells [8]. In the current study it appears to have some prognostic significance, though future prospective studies in larger cohorts will be required to confirm these findings.

Telomere length, and in particular peripheral blood telomere length, has also been widely investigated as a cancer risk factor and shorter telomeres appear to correlate with increased risk in several cancer types [16]. Telomere shortening is also likely to be involved in tumor initiation of gastrointestinal tumors [17]. Some studies of this tumor group have shown a tendency for increased risk with detection of short telomeres in blood, although small effect sizes have led to a suggestion that larger trials would be appropriate [18]. It seems logical that combining telomere length with other candidate senescence markers may be an appropriate measurement in future studies. However, use of a senescence signature as a monitoring tool in the clinical setting will also require changes in signal to be clearly correlated among diverse markers. In this respect, we found that increasing activity of NAG can be detected in response to a treatment cycle with fluoropyrimidine and the magnitude of elevation correlates with response to treatment.

Hence, greater understanding of the plasma dynamics of NAG (and other candidate markers of DNA damage and senescence) and their relation to treatment response may be of considerable importance. Correlating changes among markers will also be of interest. To address this, we have recently initiated two large prospective longitudinal studies (UK Clinical Research Network trials 12434 and 12435), also in advanced gastrointestinal adenocarcinoma patients treated with fluoropyrimidine therapy. The objective of these studies is to measure the dynamics of a range of blood-based markers of aging, senescence and DNA damage. Results of these ongoing studies should help to clarify the significance of plasma biomarker dynamics in these patients.

Conclusion

Our study provides the first evidence that plasma NAG activity associates with gastrointestinal cancer outcomes and treatment responses, supporting the concept that plasma markers of human aging and DNA damage may be clinically useful for disease monitoring. Further prospective studies will be needed to extend these results and to correlate NAG levels with other candidate senescence markers.

Future perspective

In order for the promise of stratified medicine to be fulfilled in cancer therapy a wide range of new biomarkers will be required, both to characterize disease and to sensitively monitor responses to therapy. Markers related to senescence, inflammation and DNA damage are emerging as potentially important candidates in this respect

given the intrinsic involvement of these processes in cancer progression and therapeutic responses. However, there are many potential candidates for evaluation related to these processes and no single marker definitively points to senescence. Hence, it is likely that panels of biomarkers will be required in most settings to provide reliable prediction of complex disease processes.

Markers, such as NAG, which are detectable in blood and are thus amenable to repeat sampling will be extremely valuable in the longitudinal studies, such as UK Clinical Research Network trials 12434 and 12435, that will be required to understand how molecular dynamics correlate with outcomes in the therapeutic setting. However, validation of each candidate will still require their evaluation in prospectively designed and adequately powered studies. To accelerate this process, it will be necessary to establish networks of expertise involving strong collaboration among different clinical centers and research sites to bring together the skills required to facilitate complex trials involving multiple markers.

In the UK, the Experimental Cancer Medicine Center network and the Cancer Research UK Stratified Medicine Programme provide templates at the national scale, although international networks should also be fostered. This new 'network approach' to translational medicine has the potential to increase patient access to novel therapies, as well as speeding recruitment to trials and increasing the rate of translation of new markers, which will ultimately provide the tools we require in order to better understand the heterogeneity of cancer within the individual and the population.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investi-gations involving human subjects, informed consent has been obtained from the participants involved.

EXECUTIVE SUMMARY

- *N*-acetyl-glucosaminidase (NAG) is a candidate plasma marker of DNA damage and senescence.
- Plasma NAG levels correlate with age in gastrointestinal cancer patients and in healthy individuals.
- High pretreatment NAG levels were associated with metastatic disease independently of age in gastric/esophageal patients.
- Altered NAG levels were detectable following the initiation of fluropyrimidine therapy and corresponded to treatment response.
- High pretreatment NAG levels were independently associated with poor outcome.

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Papers of special note have been highlighted as:
• of interest; •• of considerable interest

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