A metabolic phenotyping approach to understanding relationships between metabolic syndrome and breast tumour responses to chemotherapy

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Purpose: Breast cancer is associated with adverse outcomes in patients with the metabolic syndrome phenotype. To study this further, we examined the relationship between serum metabolite levels and the components of metabolic syndrome with treatment outcomes in breast cancer.

Methods: A total of 88 women with measurable breast cancer were studied; their serum metabolites as assessed by 1H nuclear magnetic resonance spectroscopy, blood pressure, lipids, glucose, body mass index and waist circumference were recorded and correlated with treatment response.

Results: We identified metabolic syndrome in approximately half of our cohort (42 patients) and observed a significant trend (P = 0.03) of increased incidence of metabolic syndrome in partial response (33.3%), stable disease (42.9%) and progressive disease groups (66.1%). High blood sugar predicted a poor response (P < 0.001). Logistic regression of metabonomic data demonstrated that high lactate (P = 0.03) and low alanine (P = 0.01) combined with high glucose (P = 0.01) were associated with disease progression.

Conclusions: Metabolic syndrome is commonly observed in metastatic breast cancer and these patients have poorer outcomes. These data, which support our previous findings, suggest that high blood glucose as part of metabolic syndrome is associated with a poor response in breast cancer. They also validate new therapeutic approaches that focus on metabolism.

Key words: biomarkers, breast cancer, diabetes, metabolic syndrome, metabolomics

introduction

Metabolic syndrome, a cluster of disorders including hypertension, type II diabetes, dyslipidemia and obesity [1], is associated with a high risk for cardiovascular disorders and a variety of other conditions [2]. Of its components, obesity is associated with cancers of oesophagus, colon, breast (postmenopausal), kidney and the endometrium [3]; metabolic and hormonal abnormalities may contribute directly or indirectly to an environment for tumour growth.

It is well known that breast cancer is associated with poorer outcomes in patients with underlying disorders, which include the components of metabolic syndrome [4]. A number of overlapping features link breast cancer with metabolic syndrome, including hyperinsulinaemia, altered sex steroid metabolism, increased oxidative stress and extraglandular estrogen production [5, 6]. A growing body of evidence indicates that breast cancer patients with obesity, insulin resistance and diabetes have a poorer prognosis and that interventions targeted towards these phenotypes can improve cancer outcomes [7, 8]. Recently, it has been shown that diabetic patients with breast cancer receiving metformin during neoadjuvant chemotherapy have higher pathologic complete response (pCR) rates compared with diabetics not receiving metformin [9], while in the Women’s Intervention Nutrition Study, women randomly allocated to a low-fat diet had an increased relapse-free survival [10]. In a further well-publicised study, women who ate more vegetables and exercised had improved survival [11].

In the adjuvant setting, we have demonstrated that in those who rapidly gained weight several metabolite levels, notably lactate and alanine, were positively associated with and prognostic for weight gain (receiver operator characteristic area under the curve > 0.77; P < 0.05) [12]. Serum metabolites were
measured using a metabolic profiling approach (metabonomics/metabolomics) based on nuclear magnetic resonance (NMR) spectroscopy [13]. While in vivo magnetic resonance imaging and spectroscopy has been widely used to localise tumours and characterise response, solution state NMR analysis of blood serum and plasma from cancer patients has been primarily focused on defining biomarkers for early detection of malignancy [14–16]; there are very few studies dealing with prognostication or response to therapy.

Tumour cells exhibit a number of significant metabolic perturbations such as the Warburg effect that are already exploited in diagnosis and therapy and that could provide a rich source of predictive biomarkers. However, in our previous study [12], the response of serum metabolite levels to treatment was independent of adjuvant/neoadjuvant status; thus, we hypothesised that the metabolic signature associated with weight gain was not the result of tumour metabolism but rather reflected an underlying phenotype with a propensity towards high adiposity, consistent with metabolic syndrome. To investigate this further, we sought for the first time to correlate the triad of metabolic syndrome, metabonomic profiles and treatment response, including their components, aiming to establish and validate the role of these factors in women with breast cancer.

**methods**

**metabolic syndrome definition**

The aim of our study was to establish whether metabolic syndrome was associated with progressive disease and the serum components of relevance within this. Previously, several definitions for metabolic syndrome were used by various investigators including criteria by the World Health Organisation, the European Group for the Study of Insulin Resistance and the National Cholesterol Education Program—Third Adult Treatment Panel. The core components of all these definitions included insulin resistance, obesity, hypertension and dyslipidemia. From 2004, a consensus definition was promulgated by the International Diabetic Federation (IDF) which comprised the presence of central obesity (waist circumference ≥ 94 cm European males and ≥ 80 cm European females) along with any of the following two factors: fasting plasma glucose ≥ 5.6 mmol/l or previously diagnosed type II diabetes, raised blood pressure (BP; systolic ≥ 130 or diastolic ≥ 85 mmHg), raised triglycerides ≥ 1.7 mmol/l, reduced high-density lipoprotein (HDL) ≥ 1.03 mmol/l or receiving treatment of these components. We used IDF criterion for diagnosing metabolic syndrome that recommended if body mass index (BMI) was ≥ 30 kg/m² then central obesity can be assumed and waist circumference need not be measured [17]. Here, the use of obesity is an obligatory component in the diagnosis of metabolic syndrome, thought to be of particular relevance in the cancer setting [18].

**patients, sample collection and metabolic parameters**

We analysed 88 postmenopausal women with measurable breast cancer who were receiving standard cytotoxic treatment of breast cancer either (i) weekly paclitaxel for 18 weeks or (ii) 5-fluorouracil/epirubicin/cyclophosphamide once every 3 weeks; appropriate ethical approval was obtained.

Pre-treatment serum samples for NMR analysis were obtained after a 12-h overnight fast and 7.5 ml was collected in a standard serum bottle. These were immediately centrifuged in the clinic at 4°C, 12 000 g for 10 min. Serum was then aliquoted into 2-ml Eppendorf tubes and immediately frozen, first at −20°C then at −80°C. Blood samples were also taken for measuring fasting glucose, complete lipid profile including HDL cholesterol and triglycerides. Patients’ weights were taken using digital scales; waist circumference was measured using a measuring tape in the mid-point between the lowest rib and the iliac crest in expiration. BMI was calculated using the standard formula weight (kg)/ height (m²). Weight was taken in the clinic before commencing treatment and was recorded during each clinical visit, taken without any heavy clothing and also without shoes. BP was taken using the same electronic meter. All measurements were made before treatment.

Patients underwent a treatment assessment scan at ~3 months, with metastatic patients undergoing computed tomography and neoadjuvant patients receiving an ultrasound of the breast and axilla; standard RECIST criteria were used to assess response to treatment [19].

**1H NMR spectroscopy of sera and spectral processing**

Serum samples were defrosted at room temperature for no longer than 20 min and 200-μl aliquots combined with 400 μl 3 mM formate (concentration standard) in saline (0.9% NaCl in 10% D₂O:90% H₂O) were centrifuged at 12 000 g for 5 min. A 550-μl aliquot of this solution was pipetted into a 5-mm NMR tube and samples were frozen at ~4°C until NMR analysis. 1H NMR spectra (CPMG, 64 ms T₂ delay) for all serum samples were collected in ~8 min on a Bruker DRX600 spectrometer (Bruker Biospin, Rheinstetten, Germany), at a frequency of 600.29 MHz and 300 K. Samples were automatically inserted into a 5-mm TXI probe and gradient shimming applied. Spectra were processed according to established protocols using in-house software written in MATLAB for automatic phasing, baseline correction and chemical shift referencing [15,20]. Representative resonances for at least 12 metabolites, previously identified as potentially associated with weight gain and chemotherapy response in our previous study, were manually integrated in MATLAB [12]. Intensities were calibrated to the formate standard (with a nominal abundance of 2) before statistical analysis.

**statistics and sample size**

We used the ‘rms’ package within the R project for our statistical analysis [21]. We examined predictors of progression (n = 36) versus stable/ responded (n = 52) via logistic regression logit(p) = a + (b₁ x x₁) + (b₂ x x₂) + ... + (bₙ x xₙ), where the xₙ are the candidate predictors glucose, HDL, triglycerides and BMI and the binary high BP was defined as BP systolic ≥ 130 mmHg or diastolic ≥ 85 mmHg. We carried out variable selection of candidate predictors within the ‘rms’ package, which uses bootstrapping to obtain a valid estimate of Somers D rank correlation, a measure of the predictive ability of the final model where a value of 0 indicates no predictive discrimination and a value of 1.0 indicates the model’s perfect separation of patients with different outcomes [22]. The candidate predictors were treated as continuous in accordance with the recommendations of Royston et al. [23], who note the loss of information with categorisation. Assessment of the predictive ability of regression models was carried out by Somers D and Schepmers explained variation. Both measures were corrected for overoptimism in estimation of predictive ability by bootstrap sampling, whereby the backward elimination is repeated on each bootstrap sample, the predictive measure recalculated and used to correct the overoptimistic value on the original full dataset. We also assessed trend in the proportion of patients with metabolic syndrome across the three outcomes groups, progression, stable and responded, using the Cochran–Armitage trend test [24]. Metabolite levels from NMR spectroscopy were initially assessed individually using the Mann–Whitney test and then collectively (lactate, glucose, isoleucine, valine and alanine) using a logistic regression model with variable selection using backward elimination with the Akaike’s information criterion and predictive ability of the final model assessed by Somers D corrected for overfitting via bootstrapping (150 samples). Odds ratios were calculated as exp(b), where
patients and metabolic syndrome

A total of 88 patients were enrolled in our study of which 44 patients (50%) had grade III cancers and remaining 41 (46.6%) and 3 (3.4%) had grade II and grade I tumours, respectively. The histopathologic characteristics of these women’s breast cancers (Table 1) demonstrates that the majority were hormone receptor positive, HER 2 negative invasive ductal cancers, as we generally expect in such a cohort (this corresponds to recently published cohorts from our hospital with similar characteristics) [27, 28].

A total of 42 patients (47.7%) were diagnosed with metabolic syndrome and 46 (52.3%) did not fulfil the criteria (Table 2). These patients underwent response assessment midway though their planned treatment. We observed 24 individuals (27.3%) with partial responses and 28 (31.8%) with stable disease (Figure 1, top). The remaining 36 women (40.9%) progressed during therapy and of these, 22 women (61.1%) had metabolic syndrome compared with 14 patients (38.9%) form the non-metabolic syndrome group who progressed during therapy (Figure 1, lower).

We further analysed those with metastatic disease only and revealed similar findings: 37 women of 75 (49.3%) had metabolic syndrome. Of these 37 individuals, 22 (59.5%) women progressed on treatment and only 12 (32.4%) had stable disease, whereas only 3 patients (8.1%) had a partial response (Figure 1C). The proportion of patients with metabolic syndrome of those who progress is stable and responded to therapy changes from 61.1% to 42.9% to 33.3 %, respectively, which was a significant trend using the Cochran–Armitage test with the 13 neoadjuvant (non-metastatic) patients, which also showed an association in patients with high plasma glucose and disease progression (P < 0.001; Table 4).

Discussion

We report to the best of our knowledge, the first prospective cohort study linking breast cancer and metabolic syndrome with response to treatment, using metabonomics to provide insights into the roles of metabolism in cancer. Previously published studies have either compared metabolic syndrome with adverse features of breast cancer or have compared individual components of metabolic syndrome with breast cancer [29–31]. We now demonstrate a significant correlation

Table 1. Histopathology from the primary tumours of the 88 patients in the study cohort

<table>
<thead>
<tr>
<th>Grade</th>
<th>N = 88 (100%)</th>
<th>Histological type</th>
<th>N = 88 (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>3 (3.4)</td>
<td>Invasive ductal (IDC)</td>
<td>77 (87.5)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>41 (46.6)</td>
<td>Invasive lobular (ILC)</td>
<td>8 (9.1)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>44 (50)</td>
<td>Medullary</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>Receptor status</td>
<td>N = 88 (100%)</td>
<td>Metastatic sites</td>
<td>N = 75 (100%)</td>
</tr>
<tr>
<td>ER/PR positive</td>
<td>64 (72.7)</td>
<td>1 site</td>
<td>40 (53.4)</td>
</tr>
<tr>
<td>ER/PR negative</td>
<td>24 (27.3)</td>
<td>2 sites</td>
<td>19 (25.3)</td>
</tr>
<tr>
<td>Her2 positive</td>
<td>34 (38.6)</td>
<td>3 sites</td>
<td>9 (12)</td>
</tr>
<tr>
<td>Her2 negative</td>
<td>54 (61.4)</td>
<td>&gt;3 sites</td>
<td>7 (9.3)</td>
</tr>
<tr>
<td>ER/PR/Her2 negative</td>
<td>18 (20.4)</td>
<td>Total metastatic patients</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neoadjuvant patients</td>
<td>13</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; PR, progesterone receptor.
between impaired glucose tolerance (fasting glucose \(\geq 5.6\) mmol/l) and disease progression in postmenopausal women receiving treatment of breast cancer. NMR spectroscopy of blood sera from the same cohort also demonstrated associations between lactate, alanine and glucose and disease progression. Both NMR and standard clinical chemistry indicated the same quantitative difference in blood glucose (14.5% difference in medians) between disease progression and non-progressive groups, providing internal cross-platform validation of our findings.

Women with metabolic syndrome had a higher probability of being non-responders to treatment than those not having these risk factors, with impaired glucose tolerance being the component of metabolic syndrome most significantly associated with disease progression. Our work highlights the possibility that metabolic profiling may add complementary information to traditional clinical evaluation about metabolic phenotypes modulating cancer chemotherapy response.

Numerous epidemiological studies have established an association between diabetes and breast cancer incidence [32, 33]. The underlying mechanism is proposed to be hyperinsulinaemia and the actions of insulin-like growth factor I [34]. Hyperinsulinaemia is associated with aromatase stimulation and inhibiting sex hormone-binding globulin, which often causes increased estrogens [35]. However, the relationship between obesity, diabetes and breast cancer is complex, and there is evidence of increased risk of breast cancer in diabetics independent of obesity [36, 37].
To date, the only study analysing breast cancer and metabolic syndrome concluded that patients with metabolic syndrome had more aggressive tumour biology [31]. Despite the differences in the study design, our findings are consistent with the overall implication that metabolic syndrome is associated with adverse outcomes. By including treatment response assessment, we have now demonstrated that metastatic patients associated with metabolic syndrome have increased resistance to standard treatments and we infer that this subpopulation will experience higher mortality and morbidity.

Our observation that serum metabolites as well as glucose, in particular lactate, are predictive of reduced response to chemotherapy is consistent with our observation that similar molecules were prognostic for weight gain during early breast cancer chemotherapy, an indicator of poorer outcomes. Lactate is increased acutely when energy demand exceeds oxidative capacity (e.g. during exercise) and both lactate and alanine are circulating sources of pyruvate, particularly from muscle (the Cori cycle). Hence, in our previous investigation, we hypothesised that lactate is elevated in obese patients due to impaired oxidative metabolism in muscle or increased energy demand or both, as observed in other studies [38, 39]. Furthermore, it has long been known that serum lactate inhibits lipolysis in adipocytes: a recent study revealed that lactate inhibits triglyceride breakdown in adipocytes via the orphan G-protein-coupled receptor GPR81 [40]. It is also possible that lactate could act directly at the tumour site reducing tumour response. High extracellular lactate promotes invasion and metastasis [41] and inhibits cytolytic activity of cytotoxic T lymphocytes [42]. The monocarboxylate transporter MCT1 is a critical factor in lactate immunomodulation and is itself an anticancer target [43, 44].

We also observed that alanine levels were negatively associated with disease progression in contrast to lactate levels, whereas both were previously reported to have a positive association to treatment and weight gain during breast cancer chemotherapy [12]. While closely related in terms of metabolism, lactate and alanine can derive from different sources, for example protein catabolism from muscle can yield alanine directly and the relative contribution of each to anabolic processes such as glucoseogenesis also differs, as shown previously [45, 46]. Whereas individual metabolite measurements were of borderline significance, the combination of glucose, alanine and lactate was significant in multivariate analysis. While these three metabolites will vary independently, they are clearly inter-related metabolically as sources/derivatives of pyruvate. It is biologically plausible that in combination, these metabolites are more informative than individual measurements alone. The large effect observed for alanine in the model might indicate that catabolism in muscle has a particular role to play in generating the correlations observed. These data also validate our previous findings [12].

Recently, the importance of glucose metabolism in cancer biology was highlighted by a finding that GLUT1, encoding glucose transporter-1, was one of three genes consistently up-regulated in the transcriptomes of paired cell lines that differed only in their KRAS or BRAF mutational status [47]. Diabetic patients with breast cancer receiving metformin and

### Table 4. Logistic regression models predicting disease progression

<table>
<thead>
<tr>
<th>Metabolic profile data (n = 56)</th>
<th>Co-efficient</th>
<th>Standard error SE</th>
<th>Odds ratio per unit of variable (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−2.77</td>
<td>1.67</td>
<td></td>
<td>0.097</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.16</td>
<td>0.52</td>
<td>3.19 (1.15–8.84)</td>
<td>0.026</td>
</tr>
<tr>
<td>Alanine</td>
<td>−9.36</td>
<td>3.67</td>
<td>8.61 × 10⁻² (6.47 × 10⁻³ −0.11)</td>
<td>0.011</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.83</td>
<td>0.32</td>
<td>2.30 (1.22–4.30)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

### Table 5. Analysis of serum metabolic profiling data with respect to treatment outcome

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Patients with disease progression, n = 21</th>
<th>Patients with no disease progression, n = 35</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>2.715</td>
<td>2.256</td>
<td>0.18</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.598</td>
<td>0.618</td>
<td>0.47</td>
</tr>
<tr>
<td>Choline</td>
<td>0.05</td>
<td>0.056</td>
<td>0.35</td>
</tr>
<tr>
<td>Valine</td>
<td>0.239</td>
<td>0.227</td>
<td>0.83</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.064</td>
<td>0.06</td>
<td>0.54</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.079</td>
<td>0.069</td>
<td>0.31</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.472</td>
<td>0.537</td>
<td>0.13</td>
</tr>
<tr>
<td>Glucose</td>
<td>6.703</td>
<td>5.854</td>
<td>0.06</td>
</tr>
<tr>
<td>D-3-hydroxy-butyrate</td>
<td>0.167</td>
<td>0.143</td>
<td>0.09</td>
</tr>
<tr>
<td>Proline/glutamate</td>
<td>0.416</td>
<td>0.322</td>
<td>0.05</td>
</tr>
<tr>
<td>Unknown 1</td>
<td>0.028</td>
<td>0.025</td>
<td>0.06</td>
</tr>
<tr>
<td>Unknown 3</td>
<td>0.075</td>
<td>0.076</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Median relative abundance and Mann–Whitney P values (uncorrected for multiple testing) are presented.
neoadjuvant chemotherapy have a higher pCR rate than those not receiving metformin [9]. By inhibiting transcription of key gluconeogenesis genes in the liver and increasing glucose uptake in skeletal muscle, metformin reduces levels of circulating glucose, increases insulin sensitivity and reduces hyperinsulinaemia associated with insulin resistance [48].

Further work will be required to establish the biologic functions of the profile we identify and the precise mechanisms associated with a poor response to systemic therapy. Such metabonomic profiles are likely to correlate better with outcome if they reflect effects on relevant pathways, and these data require external prospective validation. Healthy nutrition will help alleviate obesity, hypertension, dyslipidemia and diabetes and hence possibly breast cancer outcomes. These data are unlikely to be breast cancer specific [49]. Assessing individuals for various components of metabolic syndrome could guide us towards personalisation of treatment at the time of diagnosis and improve outcomes if appropriate healthy lifestyle modifications or drug therapies are adopted to treat their risk factors. The ability to define metabolite correlates to response highlights the potential of metabonomics to support personalised therapy, although larger prospective studies will be required to address this. In the longer term, the acknowledgement that the biology of breast cancer patients with metabolic syndrome is different from others, and the understanding of the aberrant pathways of these individuals, should lead to the evaluation of newer therapies.

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disclosure
The authors declare no conflict of interest.

references