The impact of metabolic supply lines - and the patterns between them - on the development of distant metastases in 64 women with breast cancer

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Abstract. Cancer cells upregulate their metabolism to underlie the increased malignant activity. This requires an increased amount of 'metabolic building materials', for example glucose, amino acids etc., which have the blood circulation as their principal supply lines. Targeting these metabolic supply lines, and thus the availability of metabolic building materials in the blood, may therefore carry treatment potential. A central observation is that the malignant alterations comprise great complexity and that compensatory mechanisms exist. Therefore, targeted supply lines should presumably constitute specific patterns to achieve therapeutic effect. The aim of the present study was to investigate if such patterns could be seen to correlate with the development of distant metastases. The study was conducted using a case-cohort design. In total, 64 women diagnosed with breast cancer between January 2011 and December 2015 were included. Among these, 32 had developed distant metastases and 32 had not. From a blood sample drawn at the time of diagnosis, the levels of glucose (HbA1c), glutamine, arginine and cystathionine were measured. Cox regression was applied to investigate the impact of the supply lines of these 'building materials' and specifically the patterns between them on the development of distant metastases. The results demonstrate a significant impact of the investigated metabolic supply lines, centrally in relation to interaction between them and in relation to the impact of the increased cumulated utilization of multiple supply lines simultaneously. In conclusion, the results indicated that the metabolic supply lines may impact clinical outcome, and, in this regard, the results placed a substantial emphasis on the effect of the patterns between these supply lines.

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Introduction

Cancer cells, in contrast to normal cells, are rapidly proliferating, invasive and metastasizing. To facilitate this increased malignant activity, cancer cells reprogram and upregulate their metabolism (1,2). This consequently generates an increased degree of utilization and hence need for 'metabolic building materials', for example glucose, amino acids etc. - any component participating in the concerned metabolic processes. The blood circulation constitutes the principal supply lines for these building materials, and targeting these metabolic supply lines, practically defined as the availability of the specific building materials in the blood, could therefore carry treatment potential (3).

Glucose and amino acids are among the metabolic building materials which are central to the metabolic alterations in cancer cells (4), and studies have indicated that the blood levels of these might have a clinical impact. A meta-analysis including nearly 10,000 patients diagnosed with cancer reported that hyperglycemia was associated with shorter overall survival as well as shorter disease-free survival (5), with similar results also being reported by other large studies (n=1770-301,948) (6-9). Furthermore, other studies have found an association between glucose level and the occurrence of distant metastases in different cancers (10,11). Likewise, the levels of various amino acids in the blood have been shown to associate with both metastasis occurrence and survival (12-16).

A central aspect in this context is that the processes involved in malignancy are incredibly complex. This materializes with multiple supply lines delivering various building materials to multiple different processes (4,17). Furthermore, cancer cells are metabolically flexible, which may enable them, in the event that one supply line is limited, to compensate through upregulation of another (18). Therefore, to eventually achieve therapeutic effect, presumably more supply lines should be targeted, and these should constitute patterns that are most

crucial to the malignant processes and prevents compensation from occurring (3).

Thus, the aim should be to target the metabolic supply line patterns underlying the processes essential to malignancy, for instance uncontrolled proliferation, metastasis development etc., as this would in theory compromise the delivery of the most basal components required to exert these processes. A key aspect in this strategy is that it targets the thermodynamic foundation which enables the activities that 'make a cancer a cancer' (3).

As an initial step, this study exploratively investigates whether such metabolic supply line patterns can be seen to correlate with the development of distant metastases. The examined supply lines were those of glucose, glutamine, arginine and cystathionine. They were selected because they are involved in central malignant processes and because they can be interpreted to supplement each other in these processes. Therefore, they serve the purpose of investigating the combinatorial effects of metabolic patterns. Specifically, this study investigates whether a pattern comprising the levels of glucose (HbA1c), glutamine, arginine and cystathionine in the blood can be seen to correlate with the development of distant metastases in 64 women diagnosed with breast cancer.

Materials and methods

Patient population and blood samples. The study was approved by the Danish Committees on Health Research Ethics (H-19042347). 64 women diagnosed with breast cancer between 2011 and 2015 were included in the study. Among these, 32 had developed distant metastases and 32 had not. The patients were age 50-80 years at the time of diagnosis. Tumors were either estrogen-receptor-positive (ER+) ductal carcinomas or ER+ lobular carcinomas. Exclusion criteria were 1) previous or any other current cancer, 2) neoadjuvant treatment and 3) known diabetes mellitus or any other metabolic disease. Among the patients who had developed distant metastases, the mean age was 63.9 years (SD: 8.34) while the histological distribution included 26 (81.3%) ER+ ductal carcinomas and 6 (18.8%) ER+ lobular carcinomas. Among the patients who had not developed distant metastases, the mean age was 63.5 years (SD: 7.78) while the histological distribution was 25 (78.1%) ER+ ductal carcinomas and 7 (21.9%) ER+ lobular carcinomas.

Within 2 weeks prior to surgery, a blood sample was drawn from the patients and subsequently administered and stored in the Danish Cancer Biobank (Bio- and Genome Bank, Denmark, http://rbgb.dk/cancer) as EDTA whole blood, EDTA buffy coat, EDTA serum and plasma fractions. The samples were stored at -80°C. To ensure optimal conditions for the metabolic analyses, all samples were processed and placed in the freezer within 3 h. The patients included in the study were from two locations, Herlev Hospital, Copenhagen University Hospital, Copenhagen and Rigshospitalet, Copenhagen University Hospital, Copenhagen.

Metabolic analyses. The level of glucose in the blood was measured as Hemoglobin A1c (HbA1c). EDTA whole blood samples were thawed and 20 μl of whole blood were diluted with 1,500 ml wash (HSi Hemolysis and Wash Solution-(L)) (Tosoh Bioscience, USA) and then analyzed using the Tosoh G8 (HLC-723G8) HPLC Analyzer (Tosoh Bioscience, USA). HbA1c levels were expressed in mmol/mol. The analyses were carried out at the Department of Clinical Biochemistry, Rigshospitalet.

The levels of free glutamine, arginine and cystathionine in EDTA plasma were determined using the MassTrak Amino Acid Analysis (AAA) Solution Kit (Waters Corporation, USA), an Acquity UHPLC system with a C18 BEH column (1.7 μm;2.1x150 mm) and an integrated photodiode array (PDA) detector (operating at λ=260 nm) (all from Waters Corporation, USA). 100 μl of thawed EDTA plasma was deproteinized with an equivalent amount of 10% sulfosalicylic acid with norvaline as internal standard. The samples were then thoroughly mixed followed by centrifugation at 14,000 g. 20 μl of the supernatant were alkalized with a borate buffer/NaOH solution and derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and analyzed by UHPLC-UV using a gradient of provided eluents, a column temperature of 43°C and a flowrate of 0.45 ml/min. UHPLC conditions were set according to the instructions provided by the manufacturer with specific exceptions: 1) to enhance the separation of amino acid eluting midway in the chromatogram, AccQ-Taq Ultra Eluent B (Waters Corporation, USA) was chosen instead of the MassTrak AAA Eluent B (19); 2) to enhance the separation of arginine and glycine, a steeper gradient curve between 2 and 5.5 min was applied. Concentrations were expressed in μmol/l. The analyses were carried out at the Department of Clinical Genetics, The Metabolic Laboratory, Rigshospitalet.

For the interpretation, it is important to note that during the analyses, the supply lines are represented by the blood concentrations of the metabolic building materials. The blood glucose levels are measured as HbA1c and higher HbA1c values will directly correspond to a higher circulatory availability of glucose. The interpretation of the blood levels of glutamine, arginine and cystathionine is, however, slightly more complex. The general interpretation provided is that the higher use of these materials in cancer cells can lead to a lower concentration in the blood. The observation of lower levels will therefore be presumed to be indicative of a greater consumption and thus higher utilization of the supply lines (20). Accordingly, these observations concerning the supply lines of the metabolic building materials constitute the general framework for the interpretation of the following results.

Study design and statistical analysis. The study was conducted using a case-cohort study design. The 32 patients who had developed distant metastases were selected as cases, whereas the 32 patients who had not developed distant metastases were randomly sampled as the subcohort. All 64 patients were selected/sampled from a total cohort consisting of 816 patients diagnosed with breast cancer who were potentially eligible for inclusion. The patients were followed from the day the blood sample was drawn between 2011 and 2015 until November 30, 2019. During that period, any pathologically verified distant metastasis occurring was recorded.

Cox regression was used to determine the impact of the levels of the metabolic building materials on the development of distant metastases. To account for the non-random sampling

of the cases inherent to the case-cohort design, inverse probability weighting was applied in the regressions using Lin-Ying weights.

First, regressions were performed to initially assess the impact of the supply lines for the metabolic building materials individually while adjusting for tumor histology and age. Next, a regression model was created containing the supply lines of all selected metabolic building materials, i.e., HbA1c, glutamine, arginine and cystathionine at the same time, along with tumor histology and age as covariates. In an attempt to elucidate potential pattern effects, this model was subsequently modified to include an interaction term based on a selected set of supply lines. Finally, an 'index model' was created to examine the potential impact of metabolic supply line patterns comprising the cumulated increased utilization of multiple supply lines simultaneously. In this model, an 'index value' of 0 to 4 was calculated for each of the 64 patients where 1 point is given for displaying a higher utilization of glucose, glutamine, arginine and cystathionine, respectively. Specifically, 1 point is given for displaying an HbA1c value above the third quartile, 1 point is given for displaying a glutamine value below the median, 1 point is given for displaying an arginine value below the median and 1 point is given for displaying a cystathionine value below the median. The third quartile is used for the HbA1c values since the HbA1c analysis presents an averaged value of the blood glucose concentration. For glutamine, arginine and cystathionine, the median is used since these values are less stable and more prone to be presenting fluctuations. Thus, the 'index value' is the summed value of these points and can therefore range from 0 to 4. The 'index model' then performs a Cox regression investigating the association between this 'index value' and distant metastasis development. Afterwards, a small set of variations of the model are run to further detail this association.

P-values <0.05 were considered statistically significant. All statistical analyses were performed using R statistical software version 3.6.1.

Results

In the regression analyses examining the effects of the metabolic supply lines individually, only HbA1c was significantly associated with the development of distant metastases, when adjusted for tumor histology and age. For glutamine, arginine and cystathionine, no significant association was found. Likewise, for tumor histology and age, no significant association was found (Table I).

In the regression model including the effects of the supply lines of glucose (HbA1c), glutamine, arginine and cystathionine plus tumor histology and age, at the same time, HbA1c and cystathionine appeared significantly associated with the development of distant metastases (Table II). Hence, HbA1c remained significant but with the hazard ratio increasing in this model and with a noticeably smaller P-value. Cystathionine, which was non-significant in the previous individual models, now turned significant in this combined model.

Since lower values of cystathionine and higher values of HbA1c are associated with metastasis development, respectively, they may drive the hazard in opposite directions in the regressions. This may explain why the initial individual regressions fail to demonstrate the associations presented in the latter model where adjustment is possible.

Subsequently, this model was further modified to include an interaction term based on the potential interaction between the supply lines of cystathionine and glucose (HbA1c). This interaction term was significant (Table III) and is interpreted to indicate that, if the value of cystathionine is low, the value of HbA1c becomes more critical, whereas when the value of cystathionine is high, the value of HbA1c becomes less critical. Thus, the hazard of developing distant metastases appears to be greatest in the presence of a supply line pattern where a high supply of glucose (HbA1c) is accompanied by a high supply of cystathionine.

The results from the 'index model' showed that the 'index value' was significantly associated with the development of distant metastases (Table IV). As mentioned above, the 'index value' can range from 0 to 4 and is calculated based on 1 point being given for displaying a higher utilization of glucose, glutamine, arginine and cystathionine, respectively. The 'index model' thereby approximates a significant relationship where the hazard substantially increases for any additional supply line that is subjected to increased utilization. The model thus proposes that the hazard of developing distant metastases is greatest when a high level of supplies from multiple supply lines are present at the same time.

To further detail this association, including investigating if the effect extends beyond the two already demonstrated significant supply lines, two 'variations of the index model' were created. A model investigating the effect of increased utilization of at least three of the four studied supply lines ('index value >2 points') simultaneously was created, and a model investigating the effect of increased utilization of all four of the studied supply lines ('index value >3 points') simultaneously was created (Table IV). The first variation model presented a quite noticeable hazard ratio but was nevertheless just non-significant. In the second model, a significant rather drastic hazard ratio did occur. This drastic value seems to result from the fact that simultaneous utilization of all four supply lines appeared to be present exclusively among patients who had developed distant metastases. This may, in principle, potentially convey an important observation, but it must also be maintained that ensuing reproduction would be valuable.

In addition, it is important to note that these results also seem to imply an effect of glutamine and arginine on the development of distant metastasis.

The results from these variations of the 'index model', along with the abovementioned results, are interpreted as indicative of a general tendency where a combination of a higher number of supply lines subjected to increased utilization may be associated with the development of distant metastases. However, at the same time, the results also open the possibility of potential nuances to this association.

Discussion

HbA1c appeared significantly associated with the development of distant metastases both individually and in the model including all the investigated metabolites. Furthermore, it figures as a component of the significant 'index value' in the

Table I. Development of distant metastases: Individual impact of the metabolic supply lines (adjusted for tumor histology and age).

Parameter	Hazard ratio	95% CI	P-value
HbA1c (glucose)			
HbA1c, mmol/mol	1.148	(1.001, 1.317)	0.048
Age, years	0.996	(0.925, 1.073)	0.916
Tumor histology (ER+ ILC)	0.536	(0.137, 2.097)	0.370
Glutamine			
Glutamine, μmol/l	0.996	(0.989, 1.003)	0.267
Age, years	1.018	(0.949, 1.093)	0.612
Tumor histology (ER+ ILC)	0.913	(0.241, 3.456)	0.894
Arginine			
Arginine, μmol/l	0.997	(0.973, 1.021)	0.802
Age, years	1.017	(0.947, 1.092)	0.641
Tumor histology (ER+ ILC)	0.815	(0.230, 2.892)	0.752
Cystathionine			
Cystathionine, μmol/l	0.644	(0.285, 1.454)	0.289
Age, years	1.019	(0.950, 1.093)	0.602
Tumor histology (ER+ ILC)	0.717	(0.197, 2.614)	0.614

ER+ ILC, Estrogen-receptor-positive invasive lobular carcinoma.

Table II. Development of distant metastases: Multivariate model including all metabolic supply lines (plus tumor histology and age).

Parameter	Hazard ratio	95% CI	P-value
HbA1c, mmol/mol	1.309	(1.149, 1.490)	<0.001
Glutamine, μmol/l	1.000	(0.992, 1.009)	0.925
Arginine, μmol/l	0.968	(0.927, 1.011)	0.144
Cystathionine, μmol/l	0.145	(0.032, 0.665)	0.013
Age, years	0.951	(0.872, 1.036)	0.249
Tumor histology (ER+ ILC)	0.397	(0.101, 1.567)	0.187

ER+ ILC, Estrogen-receptor-positive invasive lobular carcinoma.

Table III. Development of distant metastases: Interaction between the supply lines of glucose and cystathionine.

Parameter	Hazard ratio	95% CI	P-value
HbA1c: Cystathionine (high)	0.906	(0.738, 1.111)	0.343
HbA1c: Cystathionine (low)	1.371	(1.236, 1.521)	<0.001

Table III shows the estimated effect of HbA1c when cystathionine is 'high' or 'low'. Cystathionine was categorized based on the blood concentration into two groups, '(high)' with higher blood concentrations (representing lower utilization) and '(low)' with lower concentrations (representing higher utilization). The effect is significantly different as the P-value for the interaction term was <0.001.

'index model'. An augmented uptake and use of glucose is a central and presumably the most well-known alteration in the metabolic reprogramming occurring in cancer cells. The Warburg effect describes this change in glucose metabolism and comprises that cancer cells direct glucose through glycolysis despite oxygen being adequately available. The resulting amplified glycolytic flux provides an increased amount of intermediates available to branching pathways, which are

Table IV. Development of distant metastases: Index model and variations of the index model.

Parameter	Hazard ratio	95% CI	P-value
Index model			
Index value	1.935	(1.028, 3.640)	0.041
Variations of the index model			
Index value >2 points	3.785	(0.964, 14.864)	0.056
Index value >3 points	44.150	(10.440, 186.708)	<0.001

able to support and underlie malignant processes (4,21). For example, this may subsequently allow for an upregulation of the branching pentose phosphate pathway, which will then facilitate an increased production of NADPH to ensure sufficient antioxidant capacity. This NADPH production may function as a central mechanism enabling the development of distant metastases, since cancer cells undergoing metastatic progression need to counter and balance substantially elevated levels of reactive oxygen species (ROS) associated with this progression. This is critical because failure to mitigate these ROS levels will result in cell damage and cell death (2,22). This example illustrates how a higher availability of glucose in the blood may potentially fuel processes contributing to the development of distant metastases.

Cystathionine was significant in the regression model containing all the supply lines and is, similarly to HbA1c and the other metabolites, a part of the calculated 'index value'. Cystathionine is a sulfur-containing metabolite and functions in the transsulfuration pathway through which it is involved in the production of glutathione, which is important in balancing ROS (23). Studies have indicated that cystathionine can be imported into cells through the system x_c^- transporter via xCT to fuel glutathione synthesis (24), and, generally, that the exogenous supply can be used to counteract ROS-induced apoptosis (25). Considering the importance of ROS-mitigation in cancer cells, a higher cystathionine supply may therefore play a role in metastasis development. Moreover, the reactions related to the transsulfuration pathway can generate hydrogen sulfide (H_2S), which has been proposed to support metastatic progression by inducing epithelial-mesenchymal transition (EMT) and cell migration and VEGFA-mediated angiogenesis (26,27). Accordingly, a higher cystathionine supply may potentially fuel these reactions leading to a greater production of H_2S with the ensuing metastatic consequences. As with glucose, these are examples of how a higher availability of cystathionine in the blood may be interpreted to potentially support the processes of metastasis.

In addition to the individual effects of glucose (HbA1c) and cystathionine, respectively, the interaction term based on the interaction between the supply lines of these building materials appeared significant, thereby indicating that the risk of developing metastases is greater with a supply line pattern where a high supply of both glucose and cystathionine is present. Hence, glucose and cystathionine may act synergistically in promoting metastatic progression. Specifically, the interaction term indicated that the value of HbA1c becomes more critical, if, at the same time, the value of cystathionine is low.

One possible theoretical interpretation of this interaction is based on the contemplation that cystathionine may potentially fuel the production of glutathione, which, in order to exert its antioxidant effect, needs NADPH that can be derived from glucose. This interplay may explain that, in order for cancer cells to counter ROS levels to allow for metastatic progression, adequate supply lines for both building (cystathionine) and subsequently running (glucose) the antioxidant machinery are needed (Fig. 1).

This would be consistent with the observation that the highest risk of developing metastases arises when the supply of both glucose and cystathionine is increased. Furthermore, it would be consistent with the specific observation that the HbA1c value is more important when the cystathionine value is low. If the cystathionine value is high (indicating low utilization), adequate amounts of NADPH would be less important, as glutathione would be present in only suboptimal measures.

Another possible theoretical interpretation of the interaction is related to the potential involvement of cystathionine in generating H_2S. It has been suggested that H_2S-mediated S-sulfhydration of lactate dehydrogenase A (LDHA) can upregulate its enzymatic activity and thereby serve to accelerate glycolysis and consequently the Warburg effect (26). The accelerated Warburg effect may then promote metastatic progression by generating NADPH for ROS-mitigation, or, for instance, through the resulting lactate production which can induce cell migration, angiogenesis and reduced immune responses (28). The most effective manifestation of this effect would again be in the presence of an adequate supply of both cystathionine and glucose, since this pattern would allow for the cystathionine derived H_2S to accelerate the Warburg effect and glucose to fuel and run through it. Concordantly, the HbA1c value becomes more important when the cystathionine value is low, as glucose would be needed to ensure adequate flux through the elevated glycolysis.

The above examples are hypothesized possible interpretations of the basis for the observed interaction effect and more interpretations could be made. What remains central in this study, is that the observed interaction effect points towards the effect of patterns as a central aspect. Furthermore, this is in line with the results of the 'index model'.

The 'index model' proposes a general relationship where the greatest risk of developing distant metastases occurs during a supply line pattern where high supplies from multiple supply lines are present at the same time. This reflects the complexity of cancer metabolism with multiple metabolic processes being involved in malignant progression. Moreover, it is in accordance with an emphasis on the importance of pattern effects.

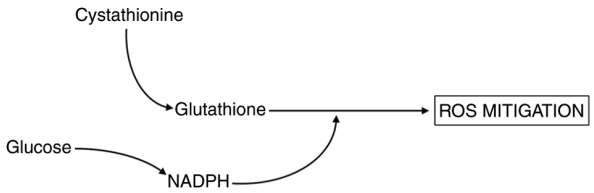

Figure 1. A hypothetical interpretation of interacting supply lines. Brief schematic representation of a possible interpretation of the basis for the interaction between the supply lines of glucose and cystathionine. ROS, reactive oxygen species.

High supplies from multiple supply lines may provide a set of favorable conditions for cancer to develop. In general, such conditions may ensure the fueling of the various processes driving different aspects of malignancy. For example, while glucose and cystathionine may facilitate ROS-mitigation, other metabolites may facilitate different functions likewise contributing to the malignant progression. Arginine may potentially fuel processes involved in immune evasion (29). Likewise, glutamine is a building material central to the metabolic alterations in cancer cells, and it has been demonstrated that highly invasive cancer cells can be characterized by upregulated glutamine metabolism (30). This observation can be extrapolated to include more potential effects derived from any additionally utilized supply of building materials. In this way, a supply line pattern comprising high supplies from multiple supply lines may potentially facilitate the employment of different malignant processes at the same time, for instance ROS-mitigation, immune evasion, invasiveness etc., which together and combined may propel disease progression. Moreover, utilization of multiple supply lines is a prerequisite for the interacting and synergistically working supply lines patterns, as described with regard to glucose and cystathionine. More of such interacting patterns obviously exist, and the simultaneous involvement of multiple supply lines serves as the foundation for the manifestation of these patterns.

Thus, a metabolic supply line pattern comprising multiple supply lines may hypothetically be able to simultaneously underlie multiple malignant processes, thereby enabling the manifestation of a cancer with more comprehensive malignant capacity.

It is important to note that the results appeared to allow for nuances in the relationship concerning the simultaneous utilization of multiple supply lines. While the general association is that increased utilization of high supplies of multiple building materials may support metastatic development, it is possible that in some cases, a low circulatory supply of a given building material might be beneficial to the cancer. For example, arginine is, while potentially being involved in immune evasion processes, coincidently also required by immune cells to maintain their optimal function (29,31). Based on this, it is possible that the supply line pattern driving malignancy most effectively might potentially also include a restricted supply of given metabolites. Nevertheless, an emphasis on the effect of patterns is maintained in both cases.

Together, the results from this study collectively seem to demonstrate that the condition of metabolic supply lines may impact outcome in patients with cancer, and furthermore, the results place a significant emphasis on the effects of patterns with regard to these supply lines.

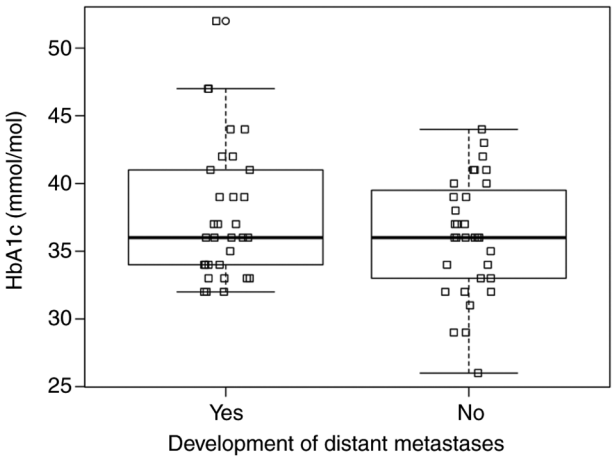

Figure 2. Metastasis development and HbA1c values. HbA1c values grouped according to whether the patients had developed distant metastases or not. HbA1c, Hemoglobin A1c.

When evaluating the results of this study, there are certain aspects and limitations that must be taken into consideration. When interpreting the results for glutamine, arginine and cystathionine, it is important to acknowledge that the observed blood concentrations are presumed to be indicative of the utilization of these materials rather than their actual availability in the blood; it does therefore not directly follow from this that intervening this availability would carry an effect. A potential effect of such an intervention could, however, for example be considered with reference to the current clinical use of asparaginase, which is used to lower circulatory asparagine in the treatment of acute lymphoblastic leukemia (ALL). Furthermore, it is important to consider that plasma generally contains cystathionine at very low concentrations, which are around the limit of quantification for the method, and the measurement of cystathionine is therefore subjected to a degree of uncertainty. However, it seems improbable that this measurement uncertainty would apply differently based on following metastasis status, and that this would manifest as significant associations. Such non-differential measurement error would be presumed to attenuate the associations (32). The inclusion of cystathionine as a binary variable in the interaction analysis and index model would also reduce this uncertainty. Moreover, common difficulties when examining amino acid concentrations in relation to cancer exist. While the general interpretation provided is that, for the amino acids, a higher use will lead to a lower concentration in the blood, it is important to note that higher concentrations can sometimes also be found to correlate with malignancy, and that generally multiple conditions may influence the association (20). Studies have demonstrated the observation that lower concentrations can reflect an increased use. For instance, this has been performed during colon cancer surgery by determining the arterio-venous difference of the amino acid levels in the tumor region and in the adjacent non-tumor region. The study generally observed a significantly greater arterio-venous difference for multiple amino acids in the tumor region, which, accordingly, was considered to result from an increased uptake by the tumor (33). However, the aforementioned difficulties should be maintained. Furthermore, it is important to note that the scale of this study is relatively small in terms of the number of included patients,

the focus on one type of cancer and the fact that few metabolic building materials are examined. Furthermore, corresponding tissue enzyme activity for the metabolites measured in the blood was not investigated. In general, the explorative nature of this work must be considered. This study should be seen as an initial step aiming to investigate any potential effect of patterns occurring between the metabolic supply lines underlying malignant processes in relation to the clinical outcome in patients with cancer. The results from this study seem to indicate that such pattern effects may in fact correlate with clinical outcome, and ensuing studies are therefore warranted.

Concerning the reflections upon a certain level of the supply lines compromising malignant capacity, a purely descriptive observation of the HbA1c values seems relevant. When the HbA1c values are divided according to whether the patients had developed distant metastases or not, it is noteworthy that, among the patients who had developed distant metastases, no HbA1c value below ~32 mmol/mol was displayed, while among the patients who had not developed distant metastases, multiple patients did present values below this point (Fig. 2).

It must be stressed that it is possible that the observation, to some extent, occurs randomly due to a relatively small number of included patients. On the contrary, the daring supposition would be to interpret this as to some degree consistent with the contemplation that an adequate supply from the metabolic supply lines may potentially be required to facilitate the processes of malignancy, and likewise, more importantly, that an intervention of the supply lines could potentially compromise these processes.

The potential of the approach presented in this study is based on the observation that the metabolic supply lines and the supply patterns between them serve as the most basic foundation for the different malignant processes of a cancer. They deliver the building blocks that are needed to perform rapid proliferation, metastatic development etc.; in other words, the different processes that intrinsically constitute and define the disease. Therefore, intervening the circulatory availability of the patterns most crucial to these processes would, in theory, target the thermodynamic foundation that facilitates the activities that 'make a cancer a cancer' (3).

In conclusion, the results from this study indicate that the levels of metabolic supply lines and the patterns between them may impact clinical outcome in patients with cancer. The results particularly place a significant emphasis on the effect of supply line patterns. Ensuing attempts to conduct investigations identifying the patterns most crucial to malignancy should therefore be made. This aim is based on the contemplation that intervening the circulatory availability of these patterns may compromise the most basic foundation for exerting these processes intrinsic to the disease.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

OA conceived the idea for the study and drafted the manuscript. EH took part in all planning. OA, EB, MC, FW, EBJ and EH designed the study and provided critical evaluation of the work. All authors provided scientific expertise within their respective fields. MC carried out the amino acid analysis. OA and EBJ performed the statistical analysis. OA and EH confirm the authenticity of all the raw data. All authors edited the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Danish Committees on Health Research Ethics (approval no. H-19042347). Informed consent was obtained from all participants. The study was performed in accordance with the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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