Letter to the Editor

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Lessons learned from site-specific sampling and biological half-life of IGFII and IIE(68-88) peptide: a case study

To the Editor,

Non-islet cell tumor induced hypoglycemia (NICTH) is a rare condition associated with mesenchymal tumors including solitary fibrous tumors (SFT) [1]. NICTH patients typically show recurrent non-ketotic hypoglycaemias, mostly at night. The presumed cause of NICTH is overexpression of the insulin-like growth factor (IGF)-II gene in the tumor resulting in overproduction of various incompletely processed, but already hormonally active proteins of the IGF system, especially pro-IGF-IIE(68-88)-peptide (IIE(68-88)) [2]. These IGFs are structurally similar to insulin and cross react with the insulin receptor (IR) [3].

Standard treatment is resecting/debulking the IGF-II producing tumor, usually leading to normalization of glucose metabolism. In patients with multiple metastases selection of the prominent IGF-II producing metastases is difficult and should be guided by biochemical evidence of hormonal production rate. In general, peripheral antecubal taken venous blood levels do not necessarily reflect tumor production-rate [2]. Both the specific location of the metastasis and differences in gene expression profiles between the primary tumor and the metastases may affect the metabolic activity of tumor cells [4, 5]. Especially in a liver with hormonally active metastases, its separate blood-supply (portal vein) and metabolism might result in high plasma levels of hormones in the draining hepatic veins which are diluted when this blood mixes with systemic blood. This necessitates site-specific venous blood-sampling in the tumor draining veins.

In this case report we describe a patient with metastatic SFT and severe hypoglycemic symptoms. To obtain insight in the contribution of liver metastases to hormone production, we measured plasma levels of IGF-II and IIE(68-88) at various sampling sites including the hepatic vein draining the largest SFT metastasis. In addition, we measured these substances in peripheral antecubal taken venous blood samples drawn at different timepoints after resection of the tumor to define the half-life and correlate these levels to tumor-size and clinical parameters.

A 34 year old male patient was diagnosed with a SFT of the pelvis in 2004, with paraneoplastic hypoglycemia with elevated IGF-II and IIE(68-88) plasma levels. He was treated with neoadjuvant chemotherapy and radiotherapy, followed by radical resection of the pelvic tumor. After being asymptomatic for years, in 2017 he developed frequent episodes of hypoglycemia. A CT-scan revealed several lesions in the lungs, liver, pelvis and ribs and a liver biopsy confirmed metastatic SFT. Despite treatment with chemotherapy,

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in 2019 progression of all metastases was shown (Supplementary Figure 1). We performed a whole-body F-18 fluorodeoxyglucose (FDG)-PET scan which did not reveal tumor uptake and thus was not discriminating in functional activity. Recurrent hypoglycemia's were controlled by 2 mg dexamethasone and 210 g cornstarch daily, resulting in weight gain and cushingoid symptoms. Laboratory values revealed slightly deranged liver tests and increased systemic serum levels of IGF-II (721 ng/mL; normal range 280–610 ng/mL) and IIE(86-88) (70 µg/L; normal range 9–27 µg/L).

Tumor volumetry was performed on the portal venous phase CT slices with 2 mm-slice reconstruction using semiautomatic Solid Lung and Liver Lesion Segmentation tools (MM Oncology package; Syngo.via; Siemens Medical, Erlangen, Germany). Only lesions with a diameter of >10 mm were included for volumetric measurements.

Based on tumor volume, the presumed largest contributor to the IGF-II and IIE(68-88) overproduction was the liver metastasis, and partial liver resection was anticipated. It could, however, not be ruled out that other tumor-sites contributed to IGF-II and IIE(68-88) production. Therefore, selective venous sampling was performed to confirm that the liver metastasis was indeed the main site of IGF-II and IIE(68-88) production. Knowledge of the halve-lives ($T_{1/2}$) of these substance is essential to interpret differences between site-specific plasma levels and systemic plasma levels. Especially a short $T_{1/2}$ will translate in a more reliable interpretation of plasma concentration differences. Literature on $T_{1/2}$ of IGF-II and IIE(68-88) is scarce [6, 7]. Of note, all publications refer to 2 papers from the late eighties investigating half-lives of IGFI and II, but not IIE(68-88) in 2 healthy individuals [8, 9]. Half-lives ranged from minutes to hours, depending on whether IGF-I and/or IGF-II are measured as free, or as a binary or tertiary structure, i.e., bound to IGFBP3 or other proteins [6]. Therefore, apart from obtaining site-specific samples we also took successive samples in order to acquire insight in the disappearance of IGF-II and IIE(68-88) after liver resection.

After being informed about the potential risks of the sampling, the patient gave consent to perform the sampling before, during and at regular time intervals after liver resection.

Preoperative sampling was performed at several sampling sites (Supplementary Figure 2). We hypothesized that the left hepatic vein, draining directly the metastasis, would contain highest IGF-II and IIE(68-88) concentrations.

Two weeks later, the patient underwent resection of liver segments 2 and 3. Intraoperatively, before the liver resection, selective sampling was performed by drawing blood from the following venous sites: left portal vein, right portal vein, left hepatic vein (tumor draining vein), right

hepatic vein and right atrium (Supplementary Figure 2). Immediately after liver resection, right atrial sampling was performed to obtain baseline values, since during a major laparotomy, patients receive large amounts of intravenous fluids leading to dilution of proteins and other constituents. In the first 3 h after closure of the abdomen, blood samples were obtained every 15 min via the central venous catheter. Afterwards, sampling was performed every hour until 6 h, followed by sampling at 8, 10, 14, 18, and 22 h post-surgery. At day 2 two samples were taken and afterwards once daily until day 5. Recovery was uneventful.

During follow-up 16 months post-surgery, signs of hypoglycemia were absent, cornstarch was stopped and dexamethasone dose was reduced step by step to 1 mg daily.

Table 1 shows the values of IGF-II and IIE(68-88) in the various sites. Compared to previous year, hormone levels increased. Insulin and IGF1 levels were suppressed or undetectable, whereas IGFBP3 levels remained stable. Preoperatively, higher IIE(68-88) but not IGF-II, levels were measured in the left hepatic vein, compared to levels in the antecubital vein or the other evaluated sites. Taking the assay coefficients of variation (CV) into account (5.2–8.3 and 8.3–11 % for IGF-II and IIE(68-88) respectively) this difference was however significant for IIE(68-88).

Intraoperative sampling 2 weeks later showed that both IGF-II and IIE(68-88) levels were highest at the intra-abdominal sampling sites as well as the central venous sampling site when the abdomen was opened. Also, higher values in the tumor draining left hepatic vein were observed. Taking the assay CVs into account this difference was significant for IGF-II but not for IIE(68-88). Calculating the gradients for IGF-II levels over the left liver lobe (outflow level in the left hepatic vein minus inflow level in the left portal vein) revealed an increase of 20 vs. 6.8 % decrease over the right liver-lobe. Similar calculations for IIE(68-88) revealed +9.8 % for the left liver-lobe and +6.7 % for the right liver-lobe.

For the calculation of the $T_{1/2}$ of both hormones, the first 300 min were omitted, because of fluid shifts due to parenteral infusions during and immediately after operation. Half-life was calculated with the following formula $C(t)=C(0)*e^{-k*t}$ with $k=\ln2/T_{1/2}$. Figure 1A shows the results of the calculation of the $T_{1/2}$ of both IGF-II and IIE(68-88). Depending on the time-interval used for calculating $T_{1/2}$ the estimated $T_{1/2}$ for IGF-II was 46 h (300–2,500 min) and 80 h (3,000–4,500 min) and for IIE(68-88) 46 and 109 h (same intervals) (Figure 1A). Supplementary Figure 3 shows the disappearance curves of IGF-II and IIE(68-88), including the first 300 min, which are likely caused by a dilution effect of intravenously administered fluids, postoperative.

Table 1: Preoperative sampling via transjugular catheterization under fluoroscopy and intraoperative sampling during surgery.

	One year earlier	IGF-II, μg/L	IIE(68-88), μg/L	IGF-I, nmol/L	Insulin, mU/L	IGFBP3, mg/L
	Antecubital vein	721	70	ND	ND	ND
	Preoperative sampling					
	Antecubital vein	1,180	153	3.0	<1.0	1.39
1	Suprahepatic inferior caval vein	1,170	158	2.8	<1.0	1.12
	Intrahepatic inferior caval vein above liver	1,120	145	3.1	<1.0	1.27
	Intrahepatic inferior caval vein below liver	1,060	162	2.8	<1.0	1.27
	Entrance of the right renal vein	1,170	149	2.9	<1.0	1.37
	Entrance of the left renal vein	1,140	155	3.1	<1.0	1.35
2	Left hepatic vein	1,180	170[a]	3.0	<1.0	1.35
	Intraoperative sampling					
4	Left portal vein	1,090	132	3.0	<1.0	ND
5	Right portal vein	1,180	135	3.0	<1.0	ND
2	Left hepatic vein (tumor draining vein)	1,310[a]	145	3.2	<1.0	ND
3	Right hepatic vein	1,100	144	3.1	<1.0	ND
	Right atrium (via central venous catheter)	1,160	126	3.1	<1.0	ND

ND, not done, numbers in column 1 correspond with the numbers in Supplementary Figure 2. [a]Higher values compared to antecubital vein or the other evaluated sites. IGF-II normal ranges: 280–610 ng/mL and IIE(86-88) normal ranges: 9.0–27 μg/L, IGF-I 10–39 nmol/L and IGFBP3 1.22–2.79 mg/L (IGF-I and IGFBP3 reference values for males, age 34).

Based on the preoperative CT scan, the tumor volume in the liver was 1,054 mL, which was 75 % of the total tumor volume of 1,406 mL including all other tumor locations. Figure 1B–D depicts tumor-volumes of the different tumor-sites (Figure 1B) and hormone levels (Figure 1D) in time. A relation was observed between hormone levels and total tumor load (Figure 1C), whereas no significant relation was found between hormone levels and specific tumor-sites (Supplementary Figure 4).

This case report describes the results of site-specific and repetitive serum sampling in a patient with symptomatic hypoglycemia's due to a disseminated SFT. Our primary aim was to identify the predominant production site by preoperative sampling via transjugular catheterization of the liver-vein draining the metastasis-containing left liver-lobe. This sampling revealed high levels, predominantly of IIE(68-88) and to a lesser extent of IGF-II. Intraoperative sampling of the left hepatic vein showed a higher IGF-II level as compared to the right hepatic vein. We found no difference for IIE(68-88) levels in the left vs. the right hepatic vein. IGF-I and insulin levels were suppressed at all timepoints. Differences between various sample sites were too small to identify the most functionally active tumor-sites.

A secondary aim was to obtain information about the $T_{1/2}$ of IGF-II and IIE(68-88) after resection of the liver metastasis. Although we realised that the other metastatic sites were also producing IGF-II and IIE(68-88), we hypothesized that after resection of the liver metastasis as presumed dominant production site, we could obtain a reliable disappearance curve. The correctness of this assumption was confirmed by the postoperative disappearance of hypoglycemic symptoms even after stopping corn starch diet and reduction of the corticosteroids.

The IGF system contains two hormones, IGF-I and IGF-II, and several intermediates. In circulation, the majority of the IGF molecules are bound to IGFBPs, of which IGFBP-3 is most prevalent. Whereas half-lives of ternary complexes consisting of IGF, IGFBP-3 and glycoprotein acid labile subunit (ALS) is over 16 h, the half-lives of binary complexes and unbound hormones are measured in minutes [8–10]. These half-lives were calculated from experiments in 2 healthy adults after bolus injection with radiolabeled IGF-II. Half-life of IIE(68-88) was, however, not investigated.

To our knowledge, this case report is first to describe site-specific sampling in a patient with NICTH aimed at providing evidence for the localization of the dominant hormone producing site. Intraoperative, but not percutaneous sampling, demonstrated a slightly higher IGFII gradient over the left liver lobe than over the (non-tumor bearing) right liver lobe. For EII(68-88), this difference was less pronounced. Although a small difference in hormone levels was found, these results provided insufficient evidence for functional dominant tumor-site localization. One explanation is the long half-life of IGFII ternary complexes. This makes site-specific sampling for analysis of IGF-II and/
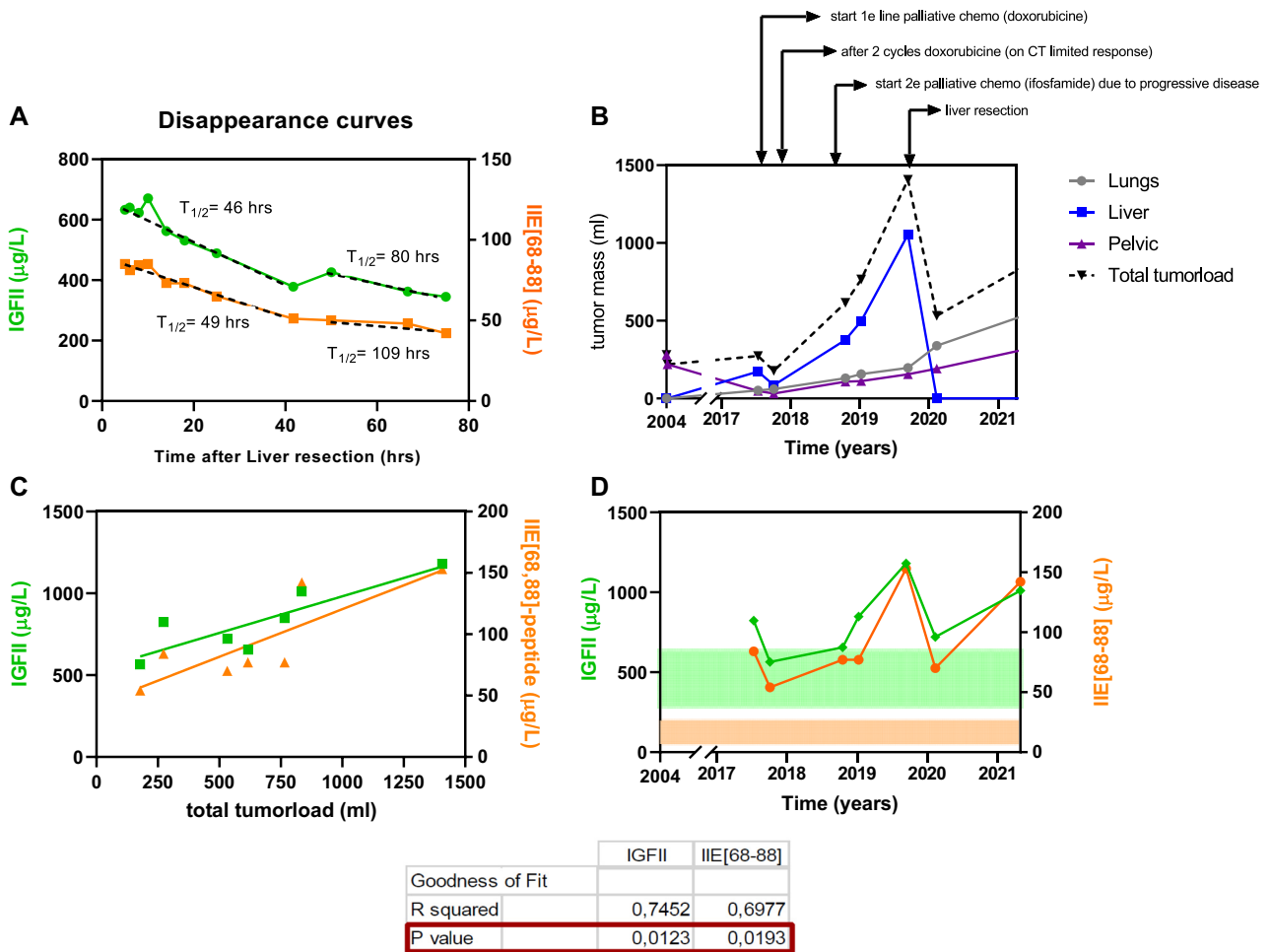
Figure 1: Hormone levels in relation to time after resection. Figure 1A depicts the half-life calculation of IGF-II (green line) and IIE(68-88) (orange line) using the following formula $C(t)=C(0)*e^{-k*t}$ with $k=\ln2/T_{1/2}$. Depending on the time interval used for calculating $T_{1/2}$ the estimated $T_{1/2}$ ranged for IGFII 46 h (300–2,500 min) and 80 h (3,000–4,500 min) and for IIE(68-88) 46 and 109 h (same intervals). (B and D) Tumor volume (B) and hormone levels (D) in time over years. Shaded areas in Figure 1D represent normal values for IGFII and IIE(68-88). (C) Correlation of tumor marker concentration and tumor volume.

or EII(68-88) in patients with NICTH of limited value. The analysis of postoperative IGF-II and EII(68-88) levels enabled us to investigate disappearance rates of these hormones after resection of the largest metastasis. Since the time-interval in which the disappearance curve for both hormones was established consisted of only a few days, the contribution of the other sites to hormone production can be considered as stable. Therefore, the decrease in hormone levels could be attributed to the removal of the liver metastasis.

Analysis of the disappearance curves of both hormones revealed roughly 2 phases. Phase 1 was characterized by a shorter half-life (46 h for IGFII and 49 h for EII(68-88)) and a phase 2 with a much longer half-life for both hormones (80 h for IGFII and 109 h for EII(68-88)).

The assays used measure total hormone levels of IGFII and EII(68-88), and do not differentiate between free or bound hormone. Therefore these different half-lives found for the different phases could well be explained by the remaining tumor and/or the different kinetics described for ternary-, and binary complexes of IGF-II and probably EII(68-88), including the different compartments (i.e., intravascular and interstitial). Size exclusion chromatography followed by immunoassay of the eluted fractions might provide more proof for this theory. Specifically, the ternary complex formation with the small IIE oligopeptide needs further investigation. Post-operative fluid shifts during the first 300 min after surgery forced us to exclude data obtained during this very early phase in our half-life calculations (see also Supplementary

Figure 3). Therefore we cannot say anything about hormone half-life in this very early phase. The disappearance rates calculated from our patient are not comparable to $T_{1/2}$ values described earlier [8, 9]. Previously, $T_{1/2}$ values were determined after a single bolus injection, whereas in our patient the elevated IGFII and EII(68-88) levels already existed for a long period of time and distribution phase had already been passed.

In conclusion, in our patient with metastatic SFT and symptomatic hypoglycemia's, site-specific sampling was of limited value in pointing out the dominant hormonally active tumor-site. Resection of the dominant tumor-site significantly reduced hypoglycemic episodes and the use of corn starch and corticosteroids. Postoperatively, hormone levels decreased significantly and half-lives for both IGFII and EII(68-88) were shown to be long, explaining the limited value of site-specific sampling.

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