Original paper

Validation of 3D power Doppler and VOCAL software in the sonographic assessment of hepatic venous flow

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Abstract

Aim: **To evaluate the reproducibility of three-dimensional power Doppler ultrasonography (3D-PDU) and the repeatability of Virtual Organ Computer-aided AnaLysis (VOCAL) software in the assessment of hepatic venous flow in ten healthy non-pregnant individuals.**
Methods: **Visualization of hepatic veins was performed using both intra- and subhepatic approaches; These examinations were repeated twice. Vascular indices were obtained for each examination in a reference point using both small and large volume samples (3 times per type of volume sample). Intraclass Correlation Coefficients and Pearson's Product-Moment Correlation Coefficient were calculated to assess reproducibility and repeatability, respectively.**
Results: **Intraclass Correlation Coefficients were more than 0.60 in small volumes, but variable in large volumes for both approaches. However, re-identification of the reference point failed in 30% using the subhepatic approach. Repeatability was high for all VOCAL analyses (Pearson's Product-Moment Correlation Coefficient > 0.98).**
Conclusions: **These results indicate reliable use of intrahepatic small volume samples in clinical application and invite to explore the role of this technology in the assessment of hepatic venous hemodynamics.**

Key words: **Hepatic blood flow, repeatability, reproducibility, three-dimensional power Doppler ultrasound, venous hemodynamics, Virtual Organ Computer-aided AnaLysis.**

Introduction

The systemic blood circulation integrates arteries and veins together with the microcirculation. The venous compartment comprises most of the systemic blood (Gelman, 2008; Pang, 2000), with the splanchnic circulation as the most important blood reservoir (Pang, 2000; Segal, 2005).

Studies of the venous compartment have gained interest in the field of obstetrics and gynaecology (Gyselaers et al., 2011; Houben et al., 2007). Throughout normal pregnancy, hepatic venous flow changes have been observed (Gyselaers, 2008; Gyselaers et al., 2011), and these patterns are different in pathologic pregnancies (Gyselaers, 2008; Gyselaers et al., 2011). Non-invasive methods such as ultrasonography are useful to study the venous compartment during pregnancy. Three-dimensional (3D) ultrasound is more accurate in volume measurements as compared to two-dimensional (2D) ultrasound and offers opportunities for clinical application in obstetrics (Lazebnik and Desser, 2007; Lee, 2007; Riccabona et al., 1996). On top of this, the application of Virtual Organ Computer-aided AnaLysis (VOCAL) software in 3D power Doppler volumes allows quantification of vascularity and blood flow, which is currently used in vascularisation studies of tumours (Parleitner et al., 1999),

placenta (Costa et al., 2010; Jones et al., 2010; Lai et al., 2010; Mercé et al., 2004), ovaries and endometrium, (Jokubkiene et al., 2006; Raine-Fenning et al., 2003) and foetus (Moron et al., 2010).

To the best of our knowledge, studies using 3D power Doppler ultrasound (3D-PDU) and VOCAL in the assessment of hepatic hemodynamics and venous blood flow in particular have not yet been published. In this study, both the use of 3D-PDU and of VOCAL software were validated in the sonographic assessment of hepatic venous hemodynamics in healthy non-pregnant individuals.

Methods

Study Population

Approval of the local ethical committee (MEC ZOL reference: 08/049) was obtained before study-onset. A heterogeneous population of ten healthy, non-pregnant subjects (3 male and 7 female) was included. After oral informed consent, each subject was sonographically examined on two separate occasions at least one week apart (exam 1 and 2). Standardization of the clinical conditions in both exams required (I) an equal time frame between last meal and exam, (II) examination at the same hour of the day and (III) after the same amount of physical exercise on both days, (IV) with identical ultrasound and Doppler settings (Raine-Fenning et al., 2008). Information about weight, blood pressure and medication use were recorded for each exam.

Sonographic exam

Sonographic exams were performed by a single operator (KT), with experience in hepatic venous Doppler flow examinations, using a 4.4 MHz probe (RAB4-8-D, Voluson E8 system, GE Healthcare, Austria). Each subject was examined in a supine position and branches of the hepatic veins were visualized from two different probe angulations, i.e. (1) the intercostal or intrahepatic approach and (2) the subhepatic approach (Figure 1). The intrahepatic approach required positioning of the probe in the transverse plane between the ribs (Figure 1a), whereas for the subhepatic approach the probe was angled towards the coronal plane underneath the thoracic chest in the right epigastrium (Figure 1b). The relevance of breath-hold during 3D-sampling was explained and demonstrated to every subject. For each method, a 2D image of the liver was stored towards definition and identification of the anatomical reference point for measuring the same hepatic vein branches on each occasion. Next to this, a 3D colour Doppler image was obtained to discriminate

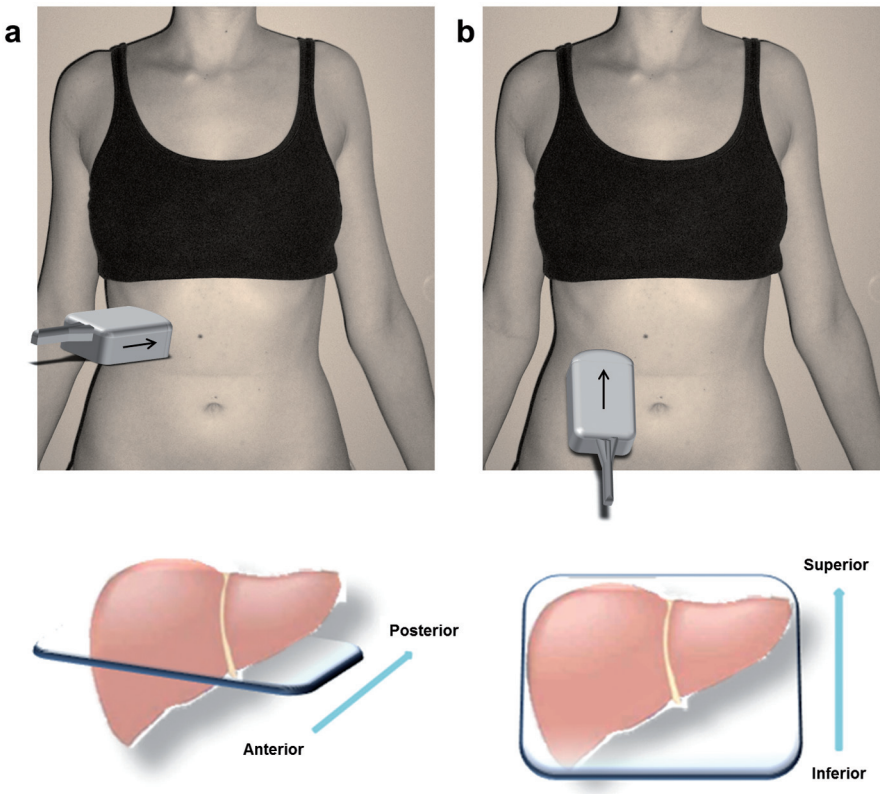

Fig. 1. — Illustration of the intrahepatic (a) and subhepatic (b) approach of the liver for three-dimensional power Doppler ultrasonography, with positioning of the probe in the transverse or coronal plane of the liver respectively.

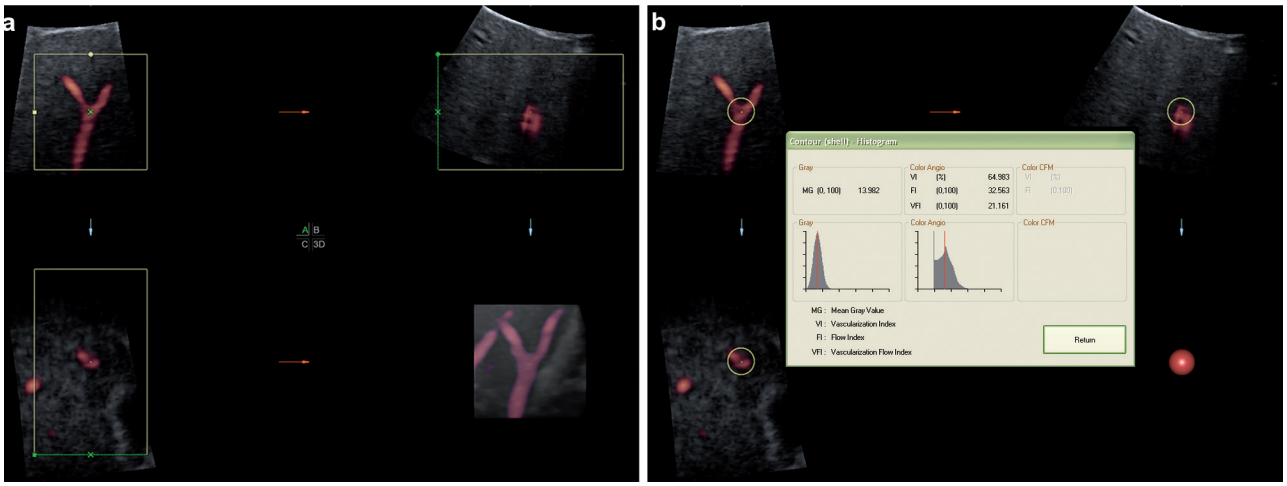

Fig. 2. — Analysis of a sphere-shaped volume in a three-dimensional power Doppler image sample around (a) a user-defined reference point (green x in upper left hand corner) with Virtual Organ Computer-aided AnaLysis (VOCAL) software. (b) Small sphere volume analysis with the corresponding histogram showing its vascularisation index (VI), flow index (FI) and vascularisation flow index (VFI).

between portal and hepatic veins in each sample. The 3D power Doppler image volumes were stored digitally on an external hard disk for vascularisation analysis. Four 3D image volumes were collected for each subject, i.e. an intrahepatic and a subhepatic image volume at exam 1, and an intrahepatic and a subhepatic image volume at exam 2.

Volume analysis

After finishing the ultrasound examination, all 3D power Doppler image volumes were analyzed using the Virtual Organ Computer-aided AnaLysis (VOCAL) software (4D View® Version 10.x, GE Healthcare, Austria). VOCAL allows for analyzing a sphere-shaped volume of the collected 3D image around a user-defined reference point (Figure 2a). Both small and large sphere volume analyses were performed for each 3D image sample by placing the contour points at the smallest (0.1 cm^3) and largest (10.0 cm^3) possible distances from each other respectively.

For each analysis, a system-specific histogram (4D View® Version 10.x, GE Healthcare, Austria) was generated, displaying the vascular indices: vascularisation index (VI; %), flow index (FI; 0-100) and vascularisation flow index (VFI; 0-100) (Figure 2b). These measured values were expressed per volume unit. All volume analyses were performed three times by the same operator (JC), each time starting from the original 3D power Doppler image. The three consecutive analyses are designated A, B and C for both the small and large volumes (Figure 3).

Statistical analysis

1) *Repeatability*

To evaluate the VOCAL software, the three consecutive volume analyses (A, B and C) within one sonographic volume of the same day were compared using the Pearson Product-Moment Correlation (PPC), i.e. A vs B, B vs C and A vs C. PPC was calculated for each parameter (VI, FI and VFI).

2) *Reproducibility*

The 3D-PDU performance, i.e. the ability to reproduce a sonographic volume around a given anatomic reference point, was evaluated using Linear Mixed Models (Verbeke and Molenberghs, 2005), which is valid even when some measurements are missing, provided the missing depends on observed rather than unobserved information. Such a model allows for population-level covariate effects (fixed effects), as well as person to person variation (random effects). For each parameter (VI, FI and VFI), Intraclass Correlation Coefficient (ICC) and standard error between exam 1 and exam 2 were calculated based on three measurements per type of sphere (small and large volumes), per type of approach (intra- and subhepatic methods), per subject (n = 10). Pearson's and Intraclass Correlation coefficients ≥ 0.80, ≥ 0.60 and < 0.60 were defined high, moderately high and low respectively.

To evaluate the most appropriate type of approach and size of volume, repeatability and reproducibility were calculated separately for intra- and

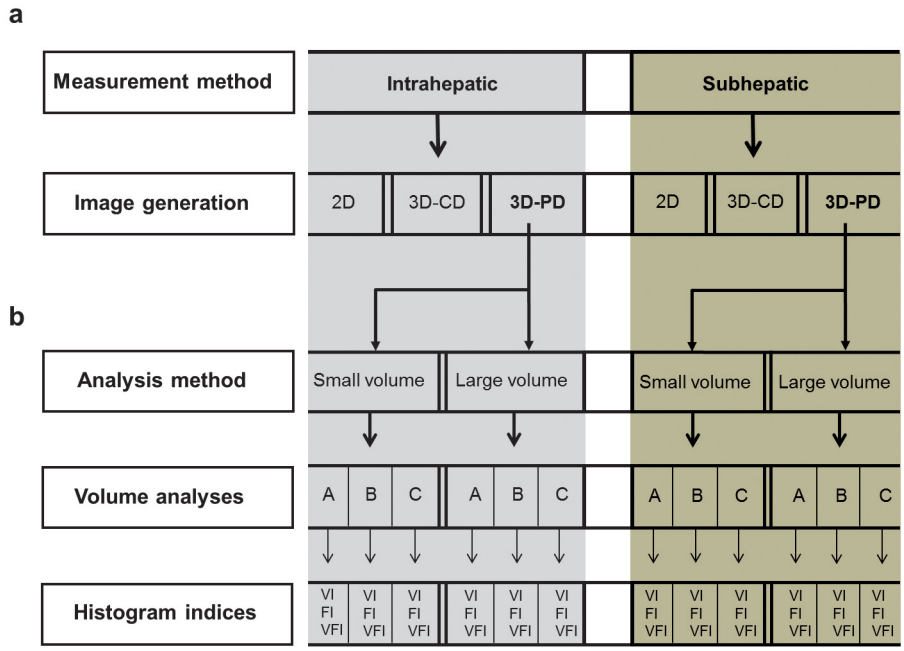

Fig. 3. — Illustration of the study protocol. (a) Intra- and subhepatic approach. Generation and storage of three images within each approach: two-dimensional (2D), three-dimensional colour (3D-CD) and 3D-power Doppler (3D-PD) ultrasound images. (b) Volume analysis. Each 3D-PD image sample was analyzed using both a small and large sphere volume. Each analysis was performed three times (A, B and C) in which vascularisation index (VI), flow index (FI) and vascularisation flow index (VFI) were measured.

subhepatic approach and for large and small sphere volumes.

Results

1) *Repeatability*

For all measurement and methods, repeatability of VOCAL-calculations within each exam was high (PPC ≥ 0.98 for A vs B, B vs C and A vs C).

2) *Reproducibility*

Reproducibility results are shown in Table I. For small sphere volumes in the intrahepatic approach, moderately high correlations (ICC between 0.60 and 0.75) were found for all parameters (VI, FI and VFI). This was not true for large sphere volumes, for which correlation coefficients were highly variable (ICC between 0.44 and 0.71).

In the subhepatic approach, identification of the reference point during exam 2 failed in 3 out of 10 cases (30%), and these data were registered as missing values. For all parameters, correlations were moderately high (ICC between 0.70 and 0.75) in small sphere volumes. This was also true for FI in large sphere volumes, but for VI and VFI very low ICC values were found (0.11 and 0.13, respectively).

Discussion

Three-dimensional-PDU and the corresponding analytical software (VOCAL) are validated in several studies regarding ovarian, endometrial and placental vascularisation (Jones et al., 2010; Lai et al., 2010; Raine-Fenning et al., 2003). However, the use of this methodology at the level of the liver has not yet been described. In this study, we assessed the reproducibility of 3D-PDU and the repeatability of the VOCAL software in ten healthy, non-pregnant subjects. In addition, we evaluated two types of probe position and sphere volumes.

Repeatability correlations of three repeated analyses within one image volume were used to evaluate the performance of the VOCAL software. These were high for both types of approach and sphere volumes (PPC ≥ 0.98). This result is identical to those reported by others (Raine-Fenning et al., 2003). The VOCAL software seems to be a reliable tool for calculating vascular indices within one image volume around a user-defined reference point. These indices may have important clinical applications in the assessment of hepatic vein hemodynamics.

The validation of the 3D-PDU examination was achieved by comparing volume analyses between two independent exams in the same subject under standardized conditions. Reproducibility correlations were moderately high (0.60 to 0.75) within

Table I. — Reproducibility of the three-dimensional power Doppler exam. For each parameter, Intraclass Correlation Coefficient and standard error (Std Error) between exam 1 and exam 2 was calculated using Linear Mixed Models for mean values of three measurements per sphere volume (small and large volumes) per type of approach (intra- and subhepatic methods) per subject. Vascularisation index (VI), flow index (FI), vascularisation flow index (VFI).

Reproducibility		Exam 1 vs Exam 2					
		Small volume (0.100 cm^3)			Large volume (10.000 cm^3)		
		VI	FI	VFI	VI	FI	VFI
Intrahepatic approach (n=10)	Correlation	0.64	0.60	0.62	0.58	0.71	0.44
	Std Error	0.20	0.21	0.21	0.22	0.17	0.27
Subhepatic approach (n=7)	Correlation	0.75	0.70	0.75	0.11	0.70	0.13
	Std Error	0.19	0.20	0.20	0.45	0.19	0.45

small sphere volumes using the intrahepatic approach. Although the subhepatic correlations were slightly higher (0.70 to 0.75), identification of the anatomic reference point failed in 30% of subjects whereas there were no failures with the intrahepatic approach. Furthermore, the subhepatic approach caused more discomfort than the intrahepatic approach, and is theoretically even more vulnerable for technical failure in late pregnancy due to the large uterine volume. Therefore, in our study set up, we consider the intrahepatic approach to be most suitable for hepatic vascularity studies. Next to this, reproducibility of VOCAL analyses within large sphere volumes samples resulted in highly variable correlations (0.11 to 0.71). This was not true for small sphere volumes, where all correlation coefficients were ≥ 0.60 (Table I).

It is likely that, in large sphere volumes, the amount of vessels captured in the 3D sample can be highly variable between two independent exams depending on the position of the ultrasound probe (Bude et al., 1994; Lazebnik and Desser, 2007; Rubin et al., 1994). Since the reproducibility correlations were lower in comparison to the repeatability correlations, 3D-PDU is more subject to variation than the VOCAL software. This can be explained by the high operator-dependence of the 3D-PDU assessment (Nelson and Pretorius, 1988), which determines image capturing (Raine-Fenning et al., 2003). Therefore we emphasize that the sonographer's use of the probe is the 'Achilles tendon' of quantitative vascularisation studies using 3D-PDU.

We conclude that three-dimensional power Doppler ultrasound and Virtual Organ Computer-aided AnaLysis software can be used to study the hepatic venous system in healthy, non-pregnant individuals. In order to obtain acceptable reproducibility and repeatability, an intrahepatic approach using small sphere volumes is preferred.

This study invites to validate three-dimensional power Doppler ultrasound and Virtual Organ Computer-aided AnaLysis in pregnant women to quantify liver vascularisation and hepatic blood flow changes during the course of normal and pathologic pregnancy.

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