Metabolomics-Based Viability Assessment of Cystic Echinococcosis Using High-Field 1H-MRS of Biopsies

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Introduction: Cystic echinococcosis (CE) or hydatid disease is caused by larval stages (metacestodes) of the cestode parasite *Echinococcus granulosus* (canine tapeworm) and is a zoonosis of worldwide distribution [1] with highest incidence in sheep breeding areas. In humans, liver and lung are the most common CE infestation sites (75% and 15%, respectively) [2]. Clinical staging of cyst development by ultrasonography (US), MRI or CT has allowed treatment options to be tailored to individual patient needs but requires accurate assessment of cyst viability on the basis of empirical correlations between cyst morphology and parasite (protoscoleces) viability, which can be determined invasively by light microscopy (LM) of biopsies [3,4]. The WHO [5] has defined six cyst stages in three clinical groups: (*i*) the "active" group includes developing cysts, unilocular (US classes CL and CE1) or multivesicular (CE2), which are usually *viable* by LM; (*ii*) the "transition" group (CE3) are cysts which are usually starting to degenerate and may be *viable* or *nonviable* by LM; (*iii*) the "inactive" group (CE4 and CE5) exhibit involution and partial or total calcification and are nearly always *nonviable*. As an alternative, we propose a multivariate metabolomics approach to cyst staging using high-field ^{1}H-MRS to examine cyst fluid or biopsy tissue. Encouraged by the initial work of Garg *et al.* [6,7], we have used 600 MHz ^{1}H-MRS to characterize cyst biopsies representing all US classes using 48 quantitative metabolic parameters.

Patients, Samples & Methods: After standardized US examination, a total of 50 cyst biopsies (47 hepatic) were aspirated from 28 patients during interventional procedures. LM examination for viability was performed, and ca. 1-mL portions were frozen for later MRS. Samples ranged from homogeneous cyst fluid to heterogeneous and highly viscous mixtures of membranous tissue and fluid. Thawed samples were transferred to 5-mm NMR tubes and viscous samples were diluted with distilled water as necessary to remove air bubbles; 50 µL TSP/D_2O was added as reference and for deuterium lock. 1D ^{1}H spectra were recorded at 600 MHz and 20 °C without sample rotation and with water suppression via presaturation (Bruker AV-600). For several samples COSY, *J*-resolved, and inverse ^{1}H/^{13}C 2D experiments were performed. This information was combined with data from the literature and on-line data bases [8] to obtain unambiguous metabolite signal assignments. Forty-four metabolites were quantitated relative to valine by signal integration and additional parameters for double bonds in fatty acid acyl chains (FA DB), triacylglycerides (TAG), *N*-acetyl hexoses (NAc) and total protein were determined. Thus, a total of 48 metabolic parameters were available for various statistical and multivariate analysis techniques (SIMCA P-11, Umetrics).

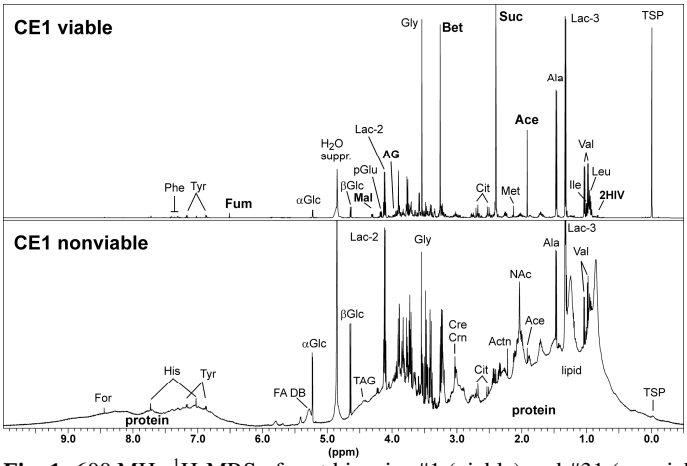

Fig. 1: 600 MHz ^{1}H-MRS of cyst biopsies #1 (viable) and #31 (nonviable).

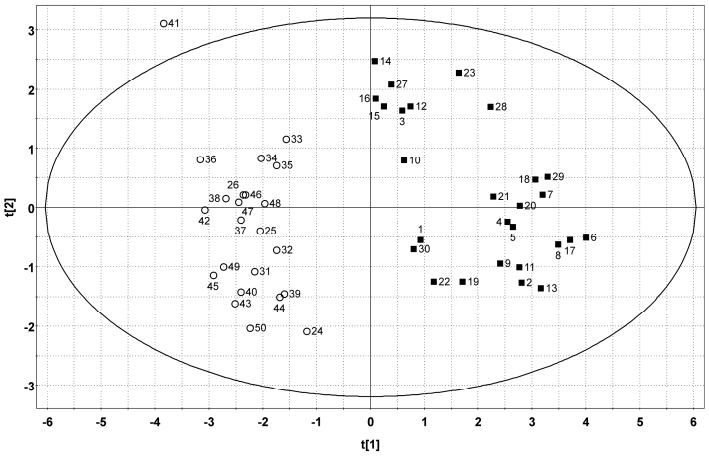

Fig. 2: PLS-DA scores plot for 50 biopsies (viable = solid squares, nonviable = open circles) using 15 metabolite parameters.

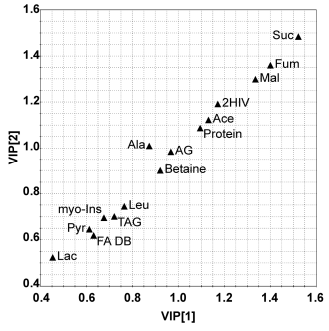

Fig. 3: 15 diagnostic metabolites.

Results & Discussion: **Fig. 1** shows ^{1}H spectra for two CE1 cysts. Key metabolites characterizing viable cysts are succinate (Suc), acetate (Ace), malate (Mal), fumarate (Fum), 2-hydroxyisovalerate (2HIV), 1,5-anhydro-D-glucitol (AG), betaine (Bet), alanine (Ala) and *myo*-inositol. These metabolites are lower in the nonviable sample, which shows elevated lactate (Lac), protein, lipid (FA DB, TAG) and NAc. **Fig. 2** presents a scores plot for principal components 1 & 2 of a partial least squares discriminant analysis (PLS-DA) for classes viable vs. nonviable, using all 50 available biopsies and the 15 most important metabolite parameters, whose rankings are shown in **Fig. 3** as the bivariate plot of the Variable Importance in the Projection (VIP) parameters. A complete separation of viable from nonviable samples is obtained over all US classes (CE1 - CE5), and the diagnostic significance [6,7] of the TCA cycle intermediates Suc, Mal, Fum is confirmed. Metabolites which are not diagnostic are glucose, glycine, citrate, creatine, choline metabolites and aromatic amino acids. A more detailed PLS-DA analysis for US classes shows that, when viable and nonviable groups are examined separately, metabolic discrimination of different US classes can be obtained and that the proposed US subclasses CE3a and CE3b are also metabolically distinguishable. Since several key metabolites should be detectable *in vivo* at field strengths >1.5 T, future noninvasive clinical applications look promising.

References:

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