Quantitative Liver Lesion Volume Determination by Nanoparticle-Based SPECT.

Veres DS, Máthé D, Futó I, Horváth I, Balázs A, Karlinger K, Szigeti K.

Published first online: 31 Aug 2013

by Springer

in Molecular Imaging and Biology

Mol Imaging Biol. 2014 Apr;16(2):167-72.; doi: 10.1007/s11307-013-0679-y

Quantitative liver lesion volume determination by nanoparticle-based SPECT

Dániel S. Veres[1], Domokos Máthé[2], Ildikó Futó[1], Ildikó Horváth[1], Ákos Balázs[3], Kinga Karlinger[4], Krisztián Szigeti[1]

1 Department of Biophysics and Radiation Biology, Semmelweis University, H-1094, Budapest, Hungary

2 CROmed Translational Research Centers, H-1047 Budapest, Hungary

3 Ist Department of Surgery, Semmelweis University, H-1094, Budapest, Hungary

4 Department of Radiology and Oncotherapy, Semmelweis University, H-1094, Budapest, Hungary

BRIEF ARTICLES

Corresponding author:

Email: szigeti.krisztian@med.semmelweis-univ.hu

Tel: +36-1-4591500/20239

Fax: +36-1-2666656

Structured Abstract

Purpose: The aim of this paper is to present a simple and quantitative data analysis method with a new potential in the application of liver SPECT imaging. We have established quantitative SPECT/CT in vivo imaging protocols for determination of liver tumor burden based on the known role of Kupffer cells in cancer of the liver.

Procedures: As it is also known that functional Kupffer cells accumulate particulate material contained in the hepatic arterial blood supply, we used radiolabeled macro-aggregated albumin particles ([^{99m}Tc]-MAA) injected intravenously to image liver disease. Quantification of cold spot liver lesion imaging was also a general objective. Methods: We examined a healthy control group (BALB/C mice, n=6) and group of induced hepatocellular carcinoma (HCC, matrilin-2 transgenic KO mice, n=9), where hepatocellular carcinoma was induced by diethylnitrosamine. As radiopharmaceutical we used [^{99m}Tc]-MAA for liver SPECT imaging in a small animal SPECT/CT system. A liver radioactivity overview map was generated. Segmentation of the liver was calculated by Otsu thresholding method. Based on the segmentation the radioactivity volume and the summarized liver activity were determined.

Results: Liver tumour burden was quantitatively determined by creating parametric data from the resulting volumetric maps. Ex vivo liver mass data were applied for the validation of in vivo measurements. An uptake with cold spots as tumors was observed in all diseased animals in SPECT/CT scans. Isotope labeled particle uptake (standardized uptake concentration – SUC) of control (median 0.33) and HCC (median 0.18) groups was significantly different ($p = 0.0015$, Mann-Whitney U test).

Conclusion: A new potential application of [^{99m}Tc]-MAA was developed and presents a simple and very effective means to quantitatively characterize liver cold spot lesions resulting from Kupffer cell dysfunctions as a consequence of tumor burden.

Keywords: hepatocellular carcinoma (HCC), SPECT/CT, quantification, particle, macro-aggregated albumin, Kupffer cell

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer mortality worldwide and it is the most common primary cancer of the liver [1]. Furthermore liver imaging is commonly undertaken in patients with tumorous anamnesis because the liver is one of the most frequently involved organs by metastatic disease either as source or target [2]. Several imaging modalities are now available for detection and characterization of tumorous lesions in the liver: ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT). The availability of combined functional/anatomical imaging modalities that integrate the benefits of visualizing tumor biology with those of high-resolution structural imaging revolutionized both clinical and pre-clinical imaging. The latter can be very important to translate new, more specific and sensitive methods of liver cancer diagnostics as well as therapeutic potential to the clinic.

In clinical practice the most commonly used liver tumor detection methods are based on anatomical imaging techniques such as X-ray CT methods and different MRI-based solutions – diffusion weighted imaging (DWI) and contrast enhanced T_1-weighted imaging and small paramagnetic iron oxide particle (SPIO) imaging. DWI tumor detection is based on the phenomenon of decreased water molecule diffusion coefficients due to increased cellularity levels in the lesion [3]. Contrast enhanced CT which is widely available and familiar with the general radiologist is the mainstay of hepatic imaging [4]. The accuracy of CT is poor at a smaller (<2 mm) tumor size therefore an MRI should be performed to fully evaluate the liver however in these cases the false positive rate increases [5].

Imaging radiolabeled compounds with SPECT and PET is often carried out to provide insight into a tumor's biological functions. Despite their high sensitivity and specificity

tomographic SPECT and PET are substantially limited by low spatial resolution and inability to provide anatomical detail [6]. Some HCC specific radiopharmaceuticals as [^{11}C]-acetate, [^{11}C]-choline and FDG are used in PET imaging [7-9]. Quantification of the metabolic rate of tumor cells and its visualization in parametric images can provide an enhanced sensitivity of diagnosis in dubious cases [10, 11]. More advantageous are those SPECT and PET imaging methods that provide hot-spot imaging where radiopharmaceuticals or radiopeptides are uptaken by tumours in a larger extent than healthy liver tissue. On the other hand, a widely available radiotracer for uniformly positive HCC cell uptake in all or even the majority of patients still can be considered missing, as [^{11}C]-labeled compounds require special radiochemistry and a nearby cyclotron, whereas [^{18}F]-choline is not showing positive contrast in tumors of all patients [12].

In the past and in current clinical practice liver radio-scintigraphy and SPECT imaging with macro-aggregated albumin ([^{99m}Tc]-MAA) or galactosyl human serum albumin ([^{99m}Tc]-GSA) [13, 14] are used to show the hepatic circulation. On the other hand these techniques have rarely been reported being used to directly detect tumorous lesions.

In hepatocellular carcinoma the number of Kupffer cells is decreased or absent in the tumor tissue [15, 16]. Previous papers have clearly demonstrated that [^{99m}Tc]-labeled nanoparticles as sulfur colloids or MAA are taken up by Kupffer cells [17]. The method of SPIO MR imaging is also based on Kupffer cell particle uptake and it shows the same diagnostic sensitivity as other MRI techniques [18].

We experimented with different clinically available radiotracers in our tumour model to show selective and positive contrast imaging of liver HCC foci in mice. Although our effort to obtain positive focal tumour contrast with several radiotracers for SPECT have not provided satisfactory results, these experiments are also briefly referred in the Methods and

Results section with the aim to provide the reader a more complete background on liver focus imaging in this model. Based on previous literature data and our experience we supposed that cold-spot imaging would have an important role in liver focus detection and follow-up. We also hypothesized that the screening of Kupffer cells using MAA in SPECT imaging would be a translatable and valid means to sensitively detect tumorous lesions in the liver as cold-spots. According to previously published in vitro and clinical studies based on the correlation between HCC and mean number of liver Kupffer cells, quantitative SPECT/CT in vivo imaging methods and image analysis could estimate tumour grade and stage of liver lesions too [16]. The aim of this article is to present a preclinical study and a simple data analysis method that could show a new potential application of MAA for clinical practice too. Therefore the often difficult interpretation of cold-spot images is made easier using the analysis described herein. Our intention was to find an appropriate, simple, and straightforward in vivo surrogate of tumor burden determination. It has paramount importance that this method could be easily executed on in vivo in situ liver data.

Material and methods

We examined a healthy control group (BALB/c, C3H, C57BL/6 mice, n=2-2-2, m=34.2 +/- 11 g) and a HCC group (cancer-prone matrilin-2 transgenic KO mice (MATN2) with chemically induced hepatocellular carcinoma, n=9, m=38.5 +/- 2 g). Hepatocellular carcinoma was induced by diethylnitrosamine on 15 days old male mice via intraperitoneal injection. The imaging was performed 4 months after induction. The presence of tumorous lesions was proved by histology in Haematoxylin-Eosin stained 10 μm thin sections (Supplementary Figure 1). All our experiments were conducted according to applicable national and European Union legislation and previously obtained the ethical approval of the

Municipal and the Local Animal Care and Use Committee. (Permission Nr. XIV-I-001/29-7/2012).

In preparation, our group has experimented with different other radiotracers to enable selective and positive contrast imaging of HCC models in mice. In previous proof-of-concept experiments in groups of n=4 mice of the same HCC-induced model we applied [^{99m}Tc]-methoxy-isobutyl-isonitrile (MIBI) – Medi-MIBI®, Medi-radiopharma Ltd, Budapest, Hungary (cca. 60 MBq iv per animal, imaging 20 minutes post injection), [^{99m}Tc]-dimercaptosuccinate (DMSA(V), cca. 80 MBq per animal, imaging 3 hours post injection – Penta-DMSA®, Medi-Radiopharma Ltd., Budapest, Hungary) and [^{201}Tl]-thallous chloride (cca. 24 MBq per animal, 4 and 24 hours post injection, IBV, Petten).

For [^{99m}Tc]-Technetium labeling, a commercially available [^{99m}Tc]-generator was used (Sorin-Drygen, Izotop, Budapest, Hungary).

Animals were anesthetized by the injection of 800 mg/kg body weight dose of 10% solution ofurethane injection intraperitoneally (ip.). We used [^{99m}Tc]-MAA (Nano-Albumon, Medi-radiopharma Ltd, Budapest, Hungary, mean diameter 200 nm, with 2-5 GBq/mg specific activity), as radiopharmaceutical for Kupffer-cell imaging (0.1-0.22 ml, 90 MBq/animal (A_{total}) iv, 2 hours post injection) for helical liver SPECT imaging (17-18 min scan time) with a voxel size of 200 um, and eXIA160 (Binitio Biomedical, Inc., Ottawa, CA) small animal contrast material (0.1 ml / 20 g iv, 2 hours post injection imaging) for liver CT imaging to enhance liver contrast (9 min scan time) with voxel size of 36 um (NanoSPECT/CT Plus Silver Upgrade, Mediso Ltd., Hungary). The segmentation of the liver images were processed first by visually delineating a Volume of Interest containing only the liver and the body as background (this process was assisted by the CT image). Thereafter Otsu thresholding method was run on this segmented SPECT image volume in VivoQuant

(inviCRO, Boston, USA) image analysis software to obtain a reliable and automatic delineation of liver versus non-liver volumes [19]. Based on the segmentation the radioactivity volume (V_{liver}) and the summarized liver activity (A_{liver}) were determined. Several semi quantitative techniques may be used to express the accumulation of radiotracers in tumours and normal tissues, including SUV (standardized uptake value). This has been described as a method of expressing biologic tissue distribution or relative concentration of radiotracers following iv administration in a variety of species [20, 21].

In our process a measurand called Standardized Uptake Concentration (SUC, Eq1.) per animal were obtained from quantified SPECT data [22].

Eq1. $$SUC = \frac{A_{liver}}{A_{total} V_{liver}}$$

After the last imaging session the animals were culled by ip. injection of 0.5 mL of euthanasia agent (T61®, Bayer, Germany) and an autopsy was performed. The livers were carefully removed by hand, placed in a filter paper sheet and then mass of each animal's liver was measured using an ABJ220-4M Precision Balance (Kern & Sohn GmbH, Balingen, Germany) with 0.1 g precision. Ex vivo liver tumor mass was used as objective parameter and was applied for the validation of in vivo measurements to correlate tumor burden measured by SPECT and in vivo really present tumor mass in the liver.

Statistical calculations

To compare control and HCC groups, Mann-Whitney U-test was applied. To check the relationship between ex vivo liver mass and segmented liver volume (ie. to account for validity of SPECT measurement segmentation), Spearman's correlation test was used.

Results

Positive tumour focus contrast SPECT imaging was not achievable in our experience by the applied radiotracers [^{99m}Tc]-MIBI, [^{99m}Tc]-DMSA(V) or [^{201}Tl]-chloride.

In Figure 1. images show the CT reconstruction of the whole body with a window to present the skeleton (left), the ^{99m}Tc isotope labeled MAA uptake in liver segmented by Otsu method 2 hours after the injection (center) and the 3D VOI created on the basis of the SPECT images. Based on this process the livers of all the animals were segmented.

In our experiment [^{99m}Tc]-MAA was detectable mainly in the liver and the bladder after two hours. The mean ratio of liver and whole body activity was 42% (SD: 6%) and 51% (SD: 5%) in the HCC and control group respectively. Figure 2. presents slices of SPECT and CT reconstructed liver images. Fig. 2A shows CT image of a healthy control without contrast material and Fig. 2B shows CT image of a HCC mouse with contrast material. SPECT image of a healthy control (Fig. 2C) shows homogenous uptake. On the other hand HCC (Fig. 2D) was acquired and the tumorous regions as several cold spots – largest on the left lobe - could be detected. (Other SPECT images of different animals are available as supplementary materials.) For quantification purposes the SUC (Eq. 1.) was defined being similar to the SUV parameter in PET experiment. We based our SUC measurements on a quantitative, previously calibrated SPECT system. This quantitative parameter helps to estimate the presence of the lesion/cancer, the volume of the lesion and the severity of the cancer.

Based on literature data we suppose relationship of in vivo defect volumes and tumor volumes [23]. The validation of liver segmentation was examined by establishing the correlation between volume via in vivo segmentation and ex vivo liver mass of HCC group. (Spearman correlation $r=0.93$, $p < 0.001$). Liver volume was plotted against liver mass as presented in Fig. 3.

In Figure 4. the box plot shows the SUC of control (median 0.33) and HCC (median 0.18) groups. The difference is statistically significant ($p = 0.0015$, Mann-Whitney U test).

Discussion and Conclusions

Based on the results of the SPECT imaging 2 hours after the radiopharmaceutical injection we can conclude that the [^{99m}Tc]-MAA is accumulated in the liver. In this time frame the MAA was not detectable in the heart and in the blood vessels. Based on literature data we suppose that [^{99m}Tc]-MAA was taken up by Kupffer cells which are the specialized resident macrophage cells of the liver [17]. The intravenously injected large sized and long circulating nanoparticles are recognized by Kupffer cells via a multitude of specialized plasma membrane receptors [24]. The mechanisms of uptake are phagocytosis and macropinocytosis mediated by complement receptors after relatively nonspecific opsonization with complement and by Fc receptors after specific opsonization with antibodies [25].

We could observe the uptake absence - cold spots - as tumorous lesions in SPECT images in all cases of diseased animals. The CT imaging has disadvantages such as longer segmentation and image processing time. Furthermore the SPECT radiopharmaceutical [^{99m}Tc]-MAA is much more economical than the available small animal CT contrast agents.

Because of the functional nature of [^{99m}Tc]-MAA SPECT we have information on cellular and even metabolic level uptake of macro-aggregates that is an indirect marker of the intact functioning of the Monocyte-Macrophage System (MMS) in the liver. Thus, the information content of these functional images is much more than identifying visible focal lesions – but that information is hidden in the data acquired. The quantitative SUC parameter could present one facet of this additional information. We hereby show the applicability of the SUV concept in quantitative SPECT studies. We found that the distribution of SUC values is

significantly different in the tumorous and control group. Therefore, the SUC value could be a useful tool to differentiate between healthy and tumorous sub-volumes of the liver SPECTs.

This study suggests that SPECT with [^{99m}Tc]-MAA can estimate both the presence and the size of tumorous lesions in the liver using a specific data analysis method. The results are promising for later preclinical and clinical practice. Nevertheless, due to the specific model of chemically induced HCC in contrast to the mostly viral origin human HCC morbidities, and in principle the sample sizes applied in our study would also warrant for thorough examination before the clinical translation.

On the other hand, our method proposed hereby is straightforward and can be applied with minimal changes of the imaging device, the patient protocol and needs only a small data analysis experience. For pre-clinical use the method would become a very powerful tool of high-content in-vivo hepatotoxicity testing after repeated or even single exposition of new chemical entities.

Acknowledgements: The authors thank dr. László Puskás and Ibolya Kiss for the animal models and dr. Miklós Kellermayer for their support. The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° HEALTH-F2-2011-278850 (INMiND), from the grant of the Hungarian Government's National Research and Development Office (NKTH-"AVINOMID"). This research was conducted in the framework of a Master Research Agreement of Semmelweis University and Mediso, Ltd and a Research Agreement of Basic Medical Sciences Centre, Semmelweis Universtiy and CROmed, Ltd.

Figures

Fig 1. NanoSPECT/CT study in healthy control and HCC mouse model with [^{99m}Tc]-MAA. The images show the CT reconstruction and the isotope labeled nanoparticle uptake in liver 2 hours after MAA injection segmented by Otsu method and the delineation of the VOI.

Fig 2. NanoSPECT/CT study in healthy control and HCC mouse model with [^{99m}Tc]-MAA. a) CT image of healthy control without contrast material b) CT image of HCC with contrast material (eXIA160) c) SPECT image of healthy control d) SPECT image of HCC.

Fig 3. The validation of segmentation is proven by correlation between volume via in vivo segmentation and ex vivo liver mass of HCC group (Spearman, $r=0.93$ $p < 0.01\%$).

Fig 4. The box plot shows the SPECT-based quasi Standardized Uptake Value (SUC) of [^{99m}Tc]-MAA of control (median 0.33) and HCC (median 0.18) groups. The difference is statistically significant ($p < 0.0015$, Mann-Whitney U test).

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

1. Altekruse SF, McGlynn KA, Reichman ME (2009) Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol. 27(9):1485-91.

2. Oliva MR, Saini S (2004) Liver cancer imaging: role of CT, MRI, US and PET. Cancer Imaging. 4 Spec No A:S42-6.

3. Qayyum A (2009) Diffusion-weighted imaging in the abdomen and pelvis: concepts and applications. Radiographics. 29(6):1797-1810.

4. Sica GT, Ji H, Ros PR (2000) CT and MR imaging of hepatic metastases. Ajr. 174(3):691-8.

5. Hammerstingl R, Huppertz A, Breuer J, Balzer T, Blakeborough A, Carter R (2008) Diagnostic efficacy of gadoxetic acid (Primovist)-enhanced MRI and spiral CT for a therapeutic strategy: comparison with intraoperative and histopathologic findings in focal liver lesions. European Radiology. 18(3):457-67.

6. Histed SN, Lindenberg ML, Mena E, Turkbey B, Choyke PL, Kurdziel KA (2012) Review of functional/anatomical imaging in oncology. Nuclear medicine communications. 33(4):349-61.

7. Delbeke D, Pinson CW. 11C-acetate: a new tracer for the evaluation of hepatocellular carcinoma (2003) J Nucl Med. 44(2):222-3.

8. Yamamoto Y, Nishiyama Y, Kameyama R, Okano K, Kashiwagi H, Deguchi A (2008) Detection of hepatocellular carcinoma using 11C-choline PET: comparison with 18F-FDG PET. J Nucl Med. 49(8):1245-8.

9. Wudel LJ, Jr., Delbeke D, Morris D, Rice M, Washington MK, Shyr Y (2003) The role of [18F]fluorodeoxyglucose positron emission tomography imaging in the evaluation of hepatocellular carcinoma. The American surgeon. 69(2):117-24; discussion 24-6.

10. Soret M, Bacharach SL, Buvat I (2007) Partial-volume effect in PET tumor imaging. J Nucl Med. 48(6):932-45.

11.Hustinx R, Paulus P, Jacquet N, Jerusalem G, Bury T, Rigo P (1998) Clinical evaluation of whole-body 18F-fluorodeoxyglucose positron emission tomography in the detection of liver metastases. Ann Oncol. 9(4):397-401.

12. Bieze M, Bennink RJ, El-Massoudi Y, Phoa SS, Verheij J, Beuers U (2013) The use of 18F-fluoromethylcholine PET/CT in differentiating focal nodular hyperplasia from hepatocellular adenoma: a prospective study of diagnostic accuracy. Nuclear medicine communications. 34(2):146-54.

13. Ziessman HA, Wahl RL, Juni JE, Gyves JE, Ensminger WD, Thrall JH (1985) The utility of SPECT for 99mTc-MAA hepatic arterial perfusion scintigraphy. Ajr. 145(4):747-51.

14. Satoh K, Yamamoto Y, Nishiyama Y, Wakabayashi H, Ohkawa M (2003) 99mTc-GSA liver dynamic SPECT for the preoperative assessment of hepatectomy. Annals of nuclear medicine. 17(1):61-7.

15.Saini S, Stark DD, Hahn PF, Bousquet JC, Introcasso J, Wittenberg J (1987) Ferrite particles: a superparamagnetic MR contrast agent for enhanced detection of liver carcinoma. Radiology. 162(1 Pt 1):217-22.

16. Liu K, He X, Lei XZ, Zhao LS, Tang H, Liu L (2003). Pathomorphological study on location and distribution of Kupffer cells in hepatocellular carcinoma. World J Gastroenterol. 9(9):1946-9.

17. George EA, Hendershott LR, Klos DJ, Donati RM (1980) Mechanism of hepatic extraction of gelatinized 99m technetium sulfur colloid. European journal of nuclear medicine. 5(3):241-5.

18. Tanimoto A, Kuribayashi S (2006) Application of superparamagnetic iron oxide to imaging of hepatocellular carcinoma. Eur J Radiol. 58(2):200-16.

19. Otsu N (1979) A Threshold Selection Method from Gray-Level Histograms. Systems, Man and Cybernetics, IEEE Transactions on 9(1):62-6.

20. Strauss LG, Conti PS (1991) The applications of PET in clinical oncology. J Nucl Med. 32(4):623-48; discussion 49-50.

21. Woodard HQ, Bigler RE, Freed B. (1975) Letter: Expression of tissue isotope distribution, J Nucl Med. 16(10):958-9.

22. Finucane CM, Murray I, Sosabowski JK, Foster JM, Mather SJ (2011) Quantitative Accuracy of Low-Count SPECT Imaging in Phantom and In Vivo Mouse Studies. International journal of molecular imaging. 2011:197381.

23. Goldfarb S, Pugh TD, Koen H, He YZ (1983) Preneoplastic and neoplastic progression during hepatocarcinogenesis in mice injected with diethylnitrosamine in infancy. Environ Health Perspect. 50:149-61.

24. Gordon S (1995) The macrophage. Bioessays. 17(11):977-86.

25. Aderem A, Underhill DM (1999) Mechanisms of phagocytosis in macrophages. Annual review of immunology. 17:593-623.