Noninvasive Molecular Imaging of Cell Death in Myocardial Infarction using $^{111}$In-GSAO


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Acute insult to the myocardium is associated with substantial loss of cardiomyocytes during the process of myocardial infarction. In this setting, apoptosis (programmed cell death) and necrosis may operate on a continuum. Because the latter is characterized by the loss of sarcolemmal integrity, we propose that an appropriately labeled tracer directed at a ubiquitously present intracellular moiety would allow non-invasive definition of cardiomyocyte necrosis. A trivalent arsenic peptide, GSAO (4-(N-(S-glutathionylacetyl)amino)phenylarsonous acid), is capable of binding to intracellular dithiol molecules such as HSP90 and filamin-A. Since GSAO is membrane impermeable and dithiol molecules abundantly present intracellularly, we propose that myocardial localization would represent sarcolemmal disruption or necrotic cell death. In rabbit and mouse models of myocardial infarction and post-infarct heart failure, we employed In-111-labelled GSAO for noninvasive radionuclide molecular imaging. $^{111}$In-GSAO uptake was observed within the regions of apoptosis seeking agent-99mTc-Annexin A5 uptake, suggesting the colocalization of apoptotic and necrotic cell death processes.

Cell death plays a central role in various cardiovascular diseases. Two morphologically distinct modes of cell death - apoptosis (a programmed process characterized by enzymatic degradation and clean removal of the cell) and necrosis (an uncontrolled process characterized by cell swelling, membrane rupture and spill of its contents) have been reported to contribute to the myocardial tissue loss. It is being increasingly realized that apoptosis and necrosis, rather than being entirely independent, may operate on a continuum, at least in response to noxious stimuli.

Numerous strategies have been proposed for the detection of cell death early after onset of ischemia within the time window amenable to intervention. Most experience for the recognition of apoptosis has been obtained with single photon emission computed tomography (SPECT) imaging using 99mTc-Annexin A5 (AA5). 99mTc-AA5 targets externalized phosphatidylserine (PS) on membranes of cells with active apoptotic signaling and the clinical feasibility of 99mTc-AA5 imaging has been demonstrated in the setting of myocardial infarction, transplant rejection and heart failure. On the other hand, several radiotracers targeting necrotic cells through membrane disruption have been developed. Notably, antimony antibody imaging has been successfully employed for the detection of myocardial necrosis associated with myocardial infarction, myocarditis, heart failure and cardiac allograft rejection. However due to technical disadvantages, the necrosis-avid radiotracers have not become popular.

4-(N-(S-glutathionylacetyl)amino)phenylarsonous acid (GSAO) labelled with fluorophores and radionuclides has been used for targeting of cell death in culture, in tumor-bearing mice, and in mice with experimental brain trauma. The principle behind GSAO cell death targeting is displayed in figure 1. GSAO is a tripeptide with a
The arsenic group binds to dithiols, which are abundantly present in the intracellular milieu and virtually absent from the extracellular space. GSAO cannot reach its intracellular target molecules in intact cells, because it is not able to negotiate across the cell membrane. Cell membrane \( \gamma \)-glutamyl transferase (GGT) is upregulated during ischemic stress and other situations, splices off GSAO’s glutamyl residue and allows cellular entrance of GSAO’s metabolite GCAO (4-(N-(S-glutathionylacetyl)amino)phenylarsenous acid). However, when radioactive or fluorescent reporter molecules are attached to glutamyl residue of GSAO it is not able to bind to GGT, rendering the molecule membrane impermeable.

After the necrotic process sets in and the sarcolemmal integrity is lost; and GSAO gains free entry to the intracellular microenvironment. Of numerous dithiol-bearing intracellular targets including filamin A, eukaryotic translation elongation factor 2, and protein disulfide isomerase (PDI), HSP90 is most widely present. HSP90 comprises approximately 2% of the intracellular protein content and increases by two-threefold in response to acute stress including ischemia.

In this study we evaluated feasibility of cell death imaging using \(^{111}\)In-GSAO in mouse and rabbit models of acute MI and a mouse model of chronic MI. In addition, a subgroup of animals, serial SPECT/CT imaging using \(^{99m}\)Tc-AA5 and \(^{111}\)In-GSAO was performed to determine the relationship between these two modes of cell death in the setting of myocardial ischemia and reperfusion. Fluorescent GSAO and AA5 were employed in another subgroup of animals for pathological characterization of the mode of cell death. In addition, \(^{111}\)In-GSCA (4-(N-(S-glutathionylacetyl)amino)benzoic acid), which is identical to \(^{111}\)In-GSAO except for replacement of the arsenic group with a carboxylic acid group, was used as a negative control compound in radionuclide experiments.

**Results**

**Fluorescence microscopic characterization of GSAO uptake.**

Uptake characteristics of GSAO were evaluated and compared with AA5 in mice with acute MI and sham-operated mice. Experimental acute MI was induced by 30-minute coronary ligation followed by 30 minutes of reperfusion. Sham procedure was identical, except that coronary ligation was not performed. Mice with acute MI \((n = 3)\) and sham-operated mice \((n = 3)\) were injected Cy5.5-labelled GSAO and Oregon Green-labeled AA5. Qualitative fluorescent microscopic analysis of heart sections showed that cardiomyocytes of sham-operated mice were positive neither for GSAO nor for AA5. In contrast, in ischemia/reperfusion-injured hearts, cardiomyocytes positive for GSAO and AA5 were observed (Fig. 2a–c). Interestingly, all GSAO-positive...
cardiomyocytes bound AA5 (Fig. 2a–c), suggesting that necrosis in the reperfused myocardium coexists with apoptotic signaling. Not all AA5-positive cardiomyocytes had taken up GSAO (Fig. 2c).

Radiolabelled GSAO imaging in acute myocardial infarction.

Acute MI was induced in rabbits and mice by coronary ligation for 40 and 30 minutes, respectively. Reperfusion was achieved by removal of the suture and 111In-GSAO was administered thirty minutes later. Three hours thereafter, in vivo micro-SPECT/micro-CT imaging was performed, animals were sacrificed and hearts were explanted. Next, ex vivo SPECT/CT and planar cardiac imaging were performed. Then, rabbit hearts were cut into ~32 small pieces and mice hearts were sectioned in three short axis slices: basal (remote area), middle (border zone) and apical (infarct). Radiotracer uptake was quantified by γ-counting, and myocardial pieces were histopathologically characterized.

In vivo SPECT/CT imaging in MI rabbits (n = 10) revealed intense uptake of 111In-GSAO in the apical area (Fig. 3a). Ex vivo SPECT/CT and planar imaging (Fig. 3b) of explanted hearts confirmed intense apical radiotracer uptake. γ-counting confirmed that the 111In-GSAO uptake in the myocardial infarct was markedly higher than the 111In-GSAO uptake in the remote region (1.10 ± 0.45%ID/g vs 0.03 ± 0.01%ID/g, P = 0.005, Fig. 3c).

To study the specificity of 111In-GSAO, the myocardial uptake of negative control compound GSCA was evaluated in acute MI rabbits (n = 5). In vivo (Fig. 3a) and ex vivo SPECT/CT imaging and planar imaging (Fig. 3b) revealed very low cardiac uptake of GSCA; only slightly increased infarct uptake was observed. This was confirmed by γ-counting; when compared with 111In-GSAO, 111In-GSCA uptake in the infarct area was significantly lower (0.07 ± 0.03%ID/g, P = 0.002, Fig 3c). Although low, GSCA uptake in the infarct area was higher than in the remote area (0.03 ± 0.01%ID/g, P = 0.043). This demonstrated that the trivalent arsenical group was not the dominant mode of cell death in myocardial infarction. The 111In-GSAO uptake in the infarct zone was higher than 111In-GSAO uptake, although the results did not reach statistical significance, due to the small sample size (1.10 ± 0.45%ID/g vs 0.47 ± 0.23%, P = 0.47). Uptake in the remote area was low for both tracers (0.03 ± 0.01%ID/g vs 0.02%ID/g, P = NS).

After γ-counting myocardial pieces from infarct, border and remote zones were histologically characterized. Representative examples of the stainings are given in figure 4a. H&E staining revealed hypereosinophilic change and contraction band necrosis in the myocardial sections from the infarct area; the morphological changes were less frequent in the border zone. The remote area was morphologically normal. The rate of apoptosis as shown by terminal deoxyribonucleotide transferase TdT-mediated nick-end labeling (TUNEL) staining (%area positive, %AP) was higher in the infarct (2.83 ± 1.42%AP) and border zone (2.55 ± 1.84%AP) than in the remote zone (0.02 ± 0.0%AP, P < 0.001). In the myocardial sections showed a direct correlation with TUNEL (r = 0.70, P < 0.001).

Figure 2 | Characterization of target binding of GSAO. Tissue sections of acute MI mice, injected fluorescently labeled GSAO and AA5. (a, b) GSAO accumulation (red) was only observed in cells with AA5-positive cell membranes (green). (c) All cells AA5-positive cells were not GSAO positive.
The non-target organ distribution of $^{111}$In-GSAO and $^{111}$In-GSCA demonstrated kidney to be the major organ of radiation burden and urine major route of excretion; all other organs revealed minimum burden for both radiotracers (Supplementary Fig. 1a). Serial blood samples from six animals revealed bi-exponential blood clearance with an initial fast component $T_{1/2a}$ of 5.3 min followed by a slower component $T_{1/2b}$ of 9.7 h; the plateau phase was approached at 15 min (Supplementary Fig. 1b).

In vivo SPECT/CT imaging in mice after acute MI ($n = 6$) demonstrated high $^{111}$In-GSAO uptake; the use of CT allowed precise localization of the radioactivity in the infarcted region of the heart (Fig. 5a,b). Specific apical uptake was confirmed by ex vivo counting of myocardial sections confirmed $^{111}$In-GSAO uptake in infarct was higher than in remote area and higher than $^{111}$In-GSCA uptake in infarct. Although low, $^{111}$In-GSCA uptake in infarct was higher than remote. Difference Between $^{111}$In-GSAO and $^{99m}$Tc-AA5 uptake did not reach significant uptake. Black horizontal lines denote Kruskal-Wallis ANOVAs + Bonferroni correction, and red lines denote Wilcoxon signed rank tests + Bonferroni correction.

Figure 3 | $^{111}$In-GSAO imaging, quantification and comparison with $^{99m}$Tc-Annexin A5 and $^{99m}$Tc-MIBI in rabbits with acute myocardial infarction. (a) In vivo SPECT (left panels), and fused SPECT/CT images (right panels) in acute MI rabbits revealed intense $^{111}$In-GSAO uptake (top) and only modest uptake of radiotracer control compound $^{111}$In-GSCA (bottom). (b) Ex vivo planar images confirmed intense $^{111}$In-GSAO uptake (top) and modest $^{111}$In-GSCA (bottom) uptake. (c) $\gamma$-counting of myocardial sections confirmed $^{111}$In-GSAO uptake in infarct was higher than in remote area and higher than $^{111}$In-GSCA uptake in infarct. Although low, $^{111}$In-GSCA uptake in infarct was higher than remote. Difference Between $^{111}$In-GSAO and $^{99m}$Tc-AA5 uptake did not reach significant uptake. Black horizontal lines denote Kruskal-Wallis ANOVAs + Bonferroni correction, and red lines denote Wilcoxon signed rank tests + Bonferroni correction. (d) Serial imaging revealed that $^{111}$In-GSAO uptake (top) was predominantly localized in the region of the $^{99m}$Tc-MIBI perfusion defect (bottom). (e) $\gamma$-counting of myocardial sections showed high $^{111}$In-GSAO uptake in sections with low MIBI uptake and vice versa. (f) A significant inverse Spearman’s correlation between uptake of $^{111}$In-GSAO and $^{99m}$Tc-MIBI was observed. (g) Serial planar imaging in acute MI rabbit demonstrates similar uptake region and higher uptake of $^{111}$In-GSAO (top) when compared with $^{99m}$Tc-AA5 (bottom). (h) $\gamma$-counting of myocardial sections showed high $^{111}$In-GSAO uptake in sections with high $^{99m}$Tc-AA5 uptake and vice versa (i). A significant Spearman’s correlation between uptake of $^{111}$In-GSAO and $^{99m}$Tc-AA5 was observed.
SPECT/CT and planar imaging (Fig. 5c). Imaging experiments revealed absence of specific $^{111}$In-GSAO uptake in control animals. $^{111}$In-GSAO uptake in the infarct area ($2.56 \pm 2.75\%$ID/g) was markedly higher than in remote myocardium ($0.48 \pm 0.22\%$ID/g, $P = 0.028$) and 20-fold higher than apical uptake in unmanipulated control animals ($n = 5, 0.13 \pm 0.02\%$ID/g; $p = 0.004$, Fig. 5d).

**Radionuclide imaging in a post-MI HF model.** $^{111}$In-GSAO uptake was also evaluated in post-MI HF mouse model, in which the LCA territory was not reperfused. Subgroups underwent imaging experiments at different time points. Also, a disease control group consisting of animals that did not undergo infarction surgery before $^{111}$In-GSAO imaging, and a radiotracer control group consisting of animals receiving negative control compound $^{111}$In-GSCA imaging at 2 weeks post-MI were used.

$^{111}$In-GSAO uptake in the HF mice was markedly lower than in AMI mice, and could not be detected by in vivo SPECT/CT imaging. Quantification of radionuclide uptake in short axis slices confirmed that infarct uptake of $^{111}$In-GSAO in mice at 2 weeks post-MI ($n = 6$) was higher than in the five disease control animals that did not undergo infarction surgery ($0.42 \pm 0.17\%$ID/g vs $0.13 \pm 0.02\%$ID/g, $P = 0.025$, Fig 6); remote uptake was not statistically different ($0.25 \pm 0.12\%$ID/g vs $0.14 \pm 0.04\%$ID/g, $P = 1.0$, Fig 6). Moreover, $^{111}$In-GSAO uptake at 2w post-MI was significantly higher than uptake of the negative control compound $^{111}$In-GSCA in the infarct and remote area ($0.04 \pm 0.02\%$ID/g, $P < 0.001$ and $0.04 \pm 0.03\%$ID/g, $P = 0.004$, respectively).

Moreover, when compared with uptake at 2 weeks post-MI, $^{111}$In-GSAO uptake showed a trend of decline at 4 weeks ($n = 6$, infarct $0.28 \pm 0.09\%$ID/g, $P = 1.0$; remote $0.22 \pm 0.04\%$ID/g, $P = 1.0$, Fig 6) and 12 weeks ($n = 8$, infarct: $0.19 \pm 0.07\%$ID/g, $P = 0.332$; remote: $0.15\% \pm 0.1\%$ID/g, $P = 1.0$, Fig 6) although differences were not statistically significant.

**Discussion**

Here we demonstrate cell death imaging in acute myocardial infarction using $^{111}$In-GSAO. The specificity of the radiotracer was confirmed by the localization of $^{111}$In-GSAO in the infarct zone as shown by $^{99m}$Tc-MIBI. This was further supported by the lack of uptake of fluorescently labeled GSAO in sham-operated animals outside of the regions directly damaged by the suture. The lack of GSAO uptake in our radionuclide studies confirmed that the trivalent arsenic group on GSAO is responsible for its targeting characteristics. The radiotracer showed favorable pharmacokinetic profile with rapid blood clearance and low background uptake in most organs.

In the 1990’s evidence of apoptotic signaling in myocardial infarction accumulated, sparking the discussion over the relative importance of the apoptotic and necrotic forms of cell death. In 2000, $^{99m}$Tc- AA5 SPECT in patients with acute myocardial infarction revealed intense uptake in the entire region of the perfusion defect. This observation was provocative as $^{99m}$Tc-AA5 was believed to identify apoptotic cell death but the infarct area was traditionally...
expected to be necrotic. It was subsequently proposed that the ischemic insult may be initiated as apoptosis but conclude with secondary necrosis. Ischemic loss of ATP production during myocardial infarction would preclude completion of the energy-dependent apoptosis program. Restoration of blood flow by reperfusion may either interrupt the apoptotic process to allow cell salvage or resume the process of apoptosis in critically damaged cells. The latter may even be augmented further by production of radical oxygen species, or intracellular calcium overload. These changes may also contribute to secondary necrosis by opening of the mitochondrial permeability transition pore. It is conceivable that the apoptotic process initiated by noxious stimuli may not follow classical picture of physiologic apoptosis observed during normal turnover of skin or mucosal cells.

The feasibility of cell death imaging using fluorescently labeled and radiolabeled GSAO has recently been demonstrated in tumor-bearing mice and mice with experimental brain trauma. GSAO accumulation was characterized by fluorescent microscopy in various cell models of apoptotic and in explanted brains and tumors after in vivo GSAO administration. GSAO was observed intracellularly and colocalized with propidium iodide and Sytox blue, standard markers of membrane disruption, which supported GSAO uptake in secondarily necrotic cells. Similarly, our fluorescent experiments showed that GSAO accumulated intracellularly and only occurred in AA5 + cells, thereby supported the notion that necrosis followed apoptotic signaling in the setting of acute myocardial infarction.

In vivo and ex vivo 111In-GSAO imaging in rabbits and mice with acute myocardial infarction showed intense dual serial imaging with 99mTc-AA5 and 111In-GSAO in rabbits after acute myocardial ischemia and reperfusion revealed the same area of uptake. At a segment level, 111In-GSAO uptake showed the same pattern as 99mTc-AA5 and a strong correlation between uptakes of the two tracers was shown. In addition, 111In-GSAO correlated with presence of Caspase-3 and TUNEL staining, classic markers of apoptosis. This shows that not only does secondary necrosis after apoptotic signaling occur in myocardial ischemia and reperfusion, it may play a dominant role. As in rabbits, high 111In-GSAO uptake was seen in mice with acute myocardial infarction. However, 111In-GSAO uptake in post-myocardial infarction HF model in mice was too low to be detected by micro-SPECT/CT imaging. The most important explanation for this is the lower rate of necrotic cell death in heart failure. It is tempting to speculate that the more benign circumstances such as lesser energy depletion and ROS cause lower transition from apoptosis to necrosis in this setting. In fact, the apoptotic process may remain suspended in chronic heart failure.

A number of imaging tracers for myocardial necrosis have been clinically evaluated. Like 111In-GSAO, most tracers exploited membrane disruption, the hallmark of necrosis as a target. Tc-99 labelled antimyosin antibody was the most widely studied necrosis tracer. Antimyosin antibody imaging has been successfully employed for the detection of myocardial necrosis associated with myocardial infarction, myocarditis, heart failure and cardiac allograft rejection. However, because of long circulation time of the radionuclide antibody, imaging was not feasible for up to 6–12 hours after administration of the agent. 99mTc-pyrophosphate on the other hand, showed maximal myocardial uptake at 24–72 hours after injection, although necrosis imaging at 3 hours post-administration was feasible. 111In-GSAO appears to have advantages over the previously evaluated cell death imaging techniques. First, 111In-GSAO requires membrane disruption, the hallmark of necrotic or late-apoptotic cell death, for reaching its intracellular targets. Therefore it does not suffer from the lack of specificity of late-gadolinium enhanced MRI. Moreover, its rapid blood clearance results in feasibility of early imaging of cell death using 111In-GSAO. This gives 111In-GSAO an advantage over 99mTc-antimyosin and 99mTc-pyrophosphate. HSP90, the main target of GSAO appears to have advantages over the previously evaluated cell death imaging techniques. First, 111In-GSAO requires membrane disruption, the hallmark of necrotic or late-apoptotic cell death, for reaching its intracellular targets. Therefore it does not suffer from the lack of specificity of late-gadolinium enhanced MRI. Moreover, its rapid blood clearance results in feasibility of early imaging of cell death using 111In-GSAO. This gives 111In-GSAO an advantage over 99mTc-antimyosin and 99mTc-pyrophosphate. HSP90, the main target of GSAO, appears to have advantages over the previously evaluated cell death imaging techniques.

Micro-SPECT/CT imaging using 111In-GSAO can be used to visualize necrotic cell death in acute myocardial infarction. The uptake in the HF model was too low to allow imaging. Because the GSAO uptake reflects membrane permeabilization, and occurs predominantly in AAS positive cells, we propose that secondary necrosis is a dominant mode of cell death in the setting of myocardial ischemia and reperfusion.
Methods
Materials. Cy5.5 GSAO and DTPA-GSAO were prepared as described previously and were obtained from Gentlest Imaging Solutions (Hazelwood, Missouri, United States). DTPA-GSAO and DTPA-GSCA were labeled with In-111 as described previously. The other used materials were of standard analytical grade.

Ethical statement. The experimental protocols followed the Guidelines for the Care and Use of Laboratory Animals established by the National Institutes of Health (NIH Publication No. 85–23, revised 1996) and was approved by the Institutional Laboratory Animal Care and Use Committees at the University of California, Irvine and University of Maastricht, Maastricht, The Netherlands.

Experimental myocardial infarction in mice. For fluorescence experiments, C57Bl/6j (Age: 3 months, weight: ~50 g, Jackson Laboratories, Sacramento, CA) and for radionuclide studies Swiss-Webster mice (Age: 3 months, weight ~50 g, Charles River, Wilmington, MA) were used. MI was induced under isoflurane anesthesia (2–3%) using a stereomicroscope (Leica MZ FL III, Leica, Switzerland) as described previously. Animals were placed on a heating pad in the supine position, intubated under direct laryngoscopy, and mechanically ventilated using a small animal respirator (tidal volume, 1.0 ml; rate, 120 breaths/min; Harvard Apparatus, Holliston, MA). After a minimum thoracotomy, the anterior descending branch of the left coronary artery was ligated with a 6.0-silk suture 3 to 4 mm below the tip of the left atrium. Successive ligation was verified by visual inspection of the LV apex for myocardial blanching, indicating interruption of coronary flow. For the induction of acute MI experiments, the suture was removed after 30 minutes to induce reperfusion. The sham operation was identical, but the coronary artery was not ligated. Acute MI and sham animals remained under anesthesia for ensuing radionuclide imaging or fluorescence experiments. MI induction (25 minutes at 50°C) and dehydration using xylene and graded series of ethanol. Tissue samples were stained with standard haematoxylin & eosin and Masson’s trichrome staining. For immunohistochemical characterization, adjacent sections were incubated with primary antibodies against Caspase-3. After washing with PBS, sections were incubated with a biotinylated secondary antibody. The presence of apoptotic cells was further evaluated using TUNEL staining as described previously. Briefly: exposed DNA fragments were labeled with biotinylated nucleotides (dTTPs) and TdT for 1 h at 37°C after blocking of endogenous peroxidase activity using 0.3%hydrogen peroxide and incubation with proteinase K.

For color reactions, sections were incubated with diaminobenzidine. For the assessment of the immunopositive area, stained tissue sections were observed under appropriate magnification (Carl Zeiss, Thornwood, New York), and the images were captured with a high-resolution digital camera (AxioCam, 1,300 × 1,030 pixels, Carl Zeiss) using Axiovision 3.1 software. Digital images were analyzed using Image-Pro Plus 5.0 (Media Cybernetics, Bethesda, Maryland).

Statistical analysis. All results are presented as the mean ± SD. In most cases, the data did not meet the assumptions of parametric tests. For consistency, non-parametric tests were used for all comparisons. Related samples were compared using Wilcoxon Signed Rank test (two groups) or Friedman’s Two Way ANOVA followed by Wilcoxon Signed Rank tests and Bonferroni Correction for pair-wise significance (more than two groups). Unrelated samples were compared using Mann Whitney U tests (two groups) or Kruskal-Wallis ANOVA followed by Mann-Whitney U tests and Bonferroni Correction for pair-wise significance (more than two groups). When the Kruskal-Wallis test revealed no significant differences among all groups, the post-hoc tests were performed. In cases where a parametric test was reported, Bonferroni correction was also applied to results of multiple Wilcoxon signed rank tests regarding the uptake of radiotracers in infant rats vs. remote areas in rabbits (fig 3C). To assess the correlation between 111In-GSAO uptake and 90Tc-AA5 uptake, 99mTc-MIBI uptake and histological findings (caspase-3 and TUNEL), Spearman’s ρ was calculated. P values of <0.05 were considered statistically significant.


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**Author contributions**

J.N. conceived the study; R.P. developed and produced the targeting agent; M.D. developed the radiolabeling protocols; C.R. conceived the mouse fluorescence experiments; H.Z. conducted the mouse imaging experiments; N.T., A.P., T.Y. and T.S. conducted rabbit experiments; J.Z. conducted and analyzed the immunohistochemistry; N.T. and H.H. performed statistical analysis; N.T., H.H. and J.N. prepared figures; N.T., H.H. and J.N. wrote the manuscript; J.N. funded the project; H.Z., A.P., R.P., T.Y., J.Z., T.I., R.S., M.D., T.S., A.K., C.R., N.N. and V.F. helped in manuscript writing and editing.

**Additional information**

Supplementary Information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: Dr. Dyszewski is an employee of Covidien Imaging Solutions, Hazelwood, Missouri. Dr. Panduranghi was an employee of Covidien when the manuscript was being conducted. The other authors claim no conflict of interest.

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