Clinical Applications of Contrast Enhanced Sonography

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Disclosures

• These applications are real, not just “academic”
• Microbubble contrast is currently not approved by FDA for non-cardiac general applications, but is approved for cardiac applications
• There is an imminent chance of approval within 1-2 years
USA is only country in world with this constraint (should be lifted within 1-2 yrs)
Microbubbles can be used “off label”

Clinical Applications

• Hepatic Masses-DDx of metastasis, focal nodular hyperplasia, hepatocellular carcinoma, hemangioma
• Renal Masses-evaluation of “indeterminate” renal mass, particularly in patients with poor renal function-DDx of renal cell carcinoma, oncocytoma
• Ovarian masses-DDx of carcinoma, fibroma, dermoid, endometrioma
• Assessment of tumor response
• Potential targeted therapy “‘theranostics’”

Research Support

• NIH/NCI grant R21 CA 125227-01
• AIUM discovery grants
• Multiple NIH/NCI grants imminent
• Philips Healthcare-software support
• Bracco-contrast and software
• Lantheus-Definity contrast

Other Applications

• Assessment of completeness of TACE, ablation
• Prostate cancer-localization prior to Bx
• Reflux-urethral, ureteric
• Splenic, renal, liver laceration 2/2 trauma
• Adnexal torsion-both depiction of vascular pedicle and “viability” of parenchyma
• Tubal patency
• Vascular complications-endovascular shunt leaks
• Breast cancer detection (Liu JUM 34:117,’15)

Tumor angiogenesis

(according to Judah Folkman, MD, PhD)
Tumor angiogenesis


Microbubble vs Gd-MRI

Kuszyk, B., AJR 177:747, 2001
MB stay intravascular, whereas Gd-MRI leaks into interstitium

Tumor Neovascularity

Increased vascularity, clustered vessels, irregular branching pattern, irregular caliber

Perfusion
Microbubbles used for Contrast Enhanced Sonography (CE-US)

Definity microbubbles

Microbubbles for US Contrast

Capillary Perfusion

Contrast Agents
• Shell
  - albumin, lipid/phospholipid or polymer
  - 5 microns
• Gas
  - air, low diffusivity gas
• Diameter
  - 2-15 microns
• Resonant frequencies - 2-10 MHz
  - Nonlinearly allows bubbles to be differentiated from surrounding tissue
• Persistence – circulate for minutes

Interaction of Ultrasound and Microbubbles

Microbubble Destruction

- At diagnostic output levels, bubble can expand a few times original radius
- Most stabilizing coatings give way, leaving a free bubble to dissolve
- After insonification at normal imaging levels, most agents are destroyed
- This can be avoided with new sensitive imaging modes, or can be used to advantage

Principles of Harmonic Imaging

Tissue and blood reflect at the fundamental frequency

- Microbubbles reflect at both the fundamental and the harmonic frequencies

Continuous vs Interval Time Delay Imaging

Continuous Imaging

- With Low MI

Interval Time Delay Imaging

- With High MI

CE-US steps

- CDS to identify region of interest
- Prepare contrast
- IV injection-contrast + 10 cc saline
  - Bolus
  - Infusion for Destruction/reperfusion
- Record in cine-loop for approx. 3 min
- Analyze enhancement parameters-time to peak, peak enhancement, washout, AUC, microvessel perfusion
Contrast Agent Preparation

Contrast Injection

A 3-µL/kg dose of Definity (~0.3 mL) injected intravenously as a bolus, followed by bolus of 10 mL of 0.9% sodium chloride solution

Destruction/Reperfusion

\[ I = \alpha(1-e^{\beta t}) \]

Hepatic Enhancement-phases

- Arterial-begins 10-20s, ends 30-45s
- Portal venous phase-begins 10-45s, ends 120s
- Late-gr than 120 s, ends 5-6 mins (300-360s)

- Sustained=likely BENIGN
- Slow washout=likely MALIGNANT
- Patterns-diffuse, nodular, peripheral

Liver Masses – Contrasted US

<table>
<thead>
<tr>
<th>Vascularity</th>
<th>Arterial Phase</th>
<th>Portal Venous Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemangioma</td>
<td>Marginal pools</td>
<td>Central progression</td>
</tr>
<tr>
<td>Focal Nodular Hyperplasia</td>
<td>Profuse stellate pattern feeding artery</td>
<td>Hypervascular with scar</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>Profuse, often dysmorphic</td>
<td>Hypervascular</td>
</tr>
<tr>
<td>Metastases</td>
<td>Variable – Usually low</td>
<td>Hypovascular</td>
</tr>
</tbody>
</table>

Focal Nodular Hyperplasia

Baseline

Arterial Phase

Portal Phase

Late Phase
Focal Nodular Hyperplasia

Early phase

Baseline

Arterial phase

Portal phase

Late phase

Focal Nodular Hyperplasia

Hemangioma

Baseline

Arterial Phase

Portal Phase

Late Phase

Early phase

Hemangioma

Late phase
Metastasis

Early phase

Late phase

Normal Liver

Arterial Phase

CE-US of Liver Masses

- PVP enhancement + in 92% benign, - in 93% malignant; sustained PVP with arterial phase nodularity and centripetal progression in 92% of hemangioma; diffuse arterial phase enhancement gr liver in 95% of FNH (Wilson, S AJR, 186:1401, 2006)
- 92-95% concordance with CT, MRI (Wilson, S Radiology 257: 24, 2010)
- Advantages-Non-toxic renal, non-ionizing

Easy to remember...

- WATCH OUT for WASH OUT!
- Rapid wash out is a sign of malignancy
- Whereas......
- Most benign lesions retain contrast in the late arterial, venous and portal venous phase

CEUS of renal masses

- Approximately 50% of people over 50 have a renal mass
- Vast majority are cysts
- Most of the solid renal masses are malignant
- Classified by Bosniak criteria-
  - B1=cyst, 0% chance of malignancy
  - B2=complex cyst, 0-5% chance of malignancy
  - B2F=increased # of septations, thick wall, 5% chance of malignancy (F=followup)
  - B3=thick septations, solid parts, 31-100% chance of malignancy
  - B4=solid, vascular mass, 100% chance of malignancy
CE-US for DDx of indeterminate renal masses
(Barr, R Radiology, 2014)

- Sensitivity=100%; specificity=95%
- PPV=95%; NPV=100%
- 5 false +’s= 3 oncocytomas, 2 B3 cysts
- Based on 721 pts, 1018 indeterminate lesions

CE-US in patients with compromised renal function

- Major advantage of CEUS over CT (potential nephrotoxicity of iodinated contrast)
- And MR (potential risk for nephrosclerosis syndrome)
- CEUS-Low cost compared to CT, MR
- Readily repeatable
- The use of CEUS could decrease the needs for both CT and MRI (diminish radiation exposure)

CE-US for ovarian Ca

- IOTA study (US O/G, 2009)-72 adnexal masses
  - AUC (0.84) better than grey scale (0.75), but less than pattern recognition (0.93)-problem DDx of borderline vs Ca-used parametric analysis, not time/intensity curves-they found CEUS could not DDx b/w borderline tumor and malignancy
- Fleischer, A et al (JUM,2008;AJR 2009)-57 adnexal masses
  - sens.=93%; spec.=96%
  - max. enhancement=90% accuracy
  - washout/AUC=100% accuracy

CEUS Image Analysis

- The area under the enhancement curve (AUC) calculated from the arrival of the contrast agent to the end of the wash-out period
- Wash-out (WO) = time between the peak intensity and the return to the baseline.
The area under the enhancement curve (AUC) calculated from the arrival of the contrast agent to the end of the wash-out period.
Peak Enhancement Time to peak

Cystadenoma

Parametric Imaging

Peak Enhancement Time to peak

Adenocarcinoma (stage II)-r ov

Serous adenocarcinoma, Stage II

Parametric Imaging

Peak Enhancement Time to peak

Adenocarcinoma (stage II)-l ov

Stage II AdenoCa

Parametric map of a serous adenocarcinoma during peak enhancement (a), time to peak (b), wAUC (c), woAUC (d), wwaAUC (e), and woR (f). Areas of tumor neovascularity in red contrast sharply with the cystic portion of the tumor shown in blue.
Adenocarcinoma (stage II) - 1 ov

Borderline mucinous cystadenocarcinoma

Borderline mucinous cystadenocarcinoma

Borderline mucinous cystadenocarcinoma

Bilateral Ov Ca (stage II)

Borderline mucinous cystadenocarcinoma

Borderline mucinous cystadenocarcinoma

T1 T2 fat-suppressed
Borderline mucinous cystadenocarcinoma

Parametric Imaging

Peak Enhancement  Time to peak

Fibroma

E_{max} = 0.41 dB
T_{1/2} = 7.5 sec
AUC = 15.3 sec^{-1}

Histology of Ovarian Masses

**Benign (n = 38)**
- endometrioma 12
- serous cystadenofibroma 7
- hemorrhagic corpus luteum 5
- mucinous cystadenoma 5
- teratoma 3
- serous cystadenoma 3
- paratubal/paraovarian cyst 2
- fibroma 1

**Borderline/Malignant (n = 12)**
- serous adenocarcinoma 6
- endometroid carcinoma 2
- breast carcinoma metastasis 2
- borderline mucinous cystadenocarcinoma 2
Enhancement parameters (n=57)

- Benign Malignant
  - Wash-Out Time, sec
    - 500
    - 1000
    - 1500
    - 2000
    - 2500
  - Area Under the Curve
  - Time to Peak, sec
- P<0.01

Diagnostic accuracy

- Sensitivity
- Specificity

Labelled MB in Animal Models of Ovarian Cancer

- Barua, A (Int J Gyn Ca; 24, 2014)-labelled
  - Alpha v beta 3 detected angiogenetic ov ca in hens
  - Lutz, A (CI Ca Res, 2014) CD276 targeted MB in mice

Procedural Applications

- s/p TACE-assess completeness of ablation
- s/p RF ablation of renal masses-assess for recurrence
- Detect abnormal sentinel nodes in breast ca

CE-US:Tumor Response

- Lassau, N Radiology 2011-Accurate assessment of HCC to antiangenic Rx-predictive of tumor response, progression-free interval, and overall survival
- Williams, R Radiology 2011-can depict changes in vascular volume earlier than RECIST
- Pei, X unpublished, 2014-Changes in cervical Ca with Rx

Good responder pre-treatment

- Volume = 19.1 cm³
Patient #1 Good Responder

Bolus sequence

Destruction-reperfusion sequence

Baseline Day 15

<table>
<thead>
<tr>
<th>Volume, cm³</th>
<th>Baseline</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.3</td>
<td>19.3</td>
<td>19.6</td>
</tr>
<tr>
<td>Contrast Enhancement, dB</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Microvascular density, dB</td>
<td>22.7</td>
<td>13.7</td>
</tr>
<tr>
<td>Blood flow velocity, 1/sec</td>
<td>1.1</td>
<td>0.7</td>
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Patient # 2 Good Responder

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day 15</th>
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</thead>
<tbody>
<tr>
<td>Volume, cm³</td>
<td>9.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Contrast Enhancement, dB</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Microvascular density, dB</td>
<td>21.5</td>
<td>9.4</td>
</tr>
<tr>
<td>Blood flow velocity, 1/sec</td>
<td>0.52</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Good responder @ d0, d15

Bolus sequence

Destruction-reperfusion sequence

Patient # 2 Good Responder

Bolus sequence

Destruction-reperfusion sequence

Good responder

Bolus sequence

Destruction-reperfusion sequence

Good responder

Bolus sequence

Destruction-reperfusion sequence
**Good responder**

<table>
<thead>
<tr>
<th>Bolus sequence</th>
<th>Destruction-reperfusion sequence</th>
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**Patient #3 Poor Responder**

<table>
<thead>
<tr>
<th>Bolus sequence</th>
<th>Destruction-reperfusion sequence</th>
</tr>
</thead>
</table>

**Poor responder @ d0, d15**

**Changes with Rx (c/o A. Lyshchik)**

**Barriers Affecting Rx**

Normalization of CEUS

Poor responder (bolus)

Poor responder (perfusion)

Fair Responder (bolus)

Good Responder (perfusion)
Excellent Responder (perfusion)

Lots of unanswered ?’s

- What defines good, fair, poor responders?
  Lassau-40% reduction in AUC
- How does this correlate with clinical response, survival?
- Multiple lesions?
- Areas of necrosis?
- Rx to decrease interstitial pressures, improve hypoxic areas

Markers for endothelial cell surface

- Inflammation (p-selectin)
- Thrombosis (alb3 integrin)
- Angiogenesis (VEGF2)

Future Opportunities and Studies

- Perfusion quantification
- Angiogenesis/anti-angiogenesis monitoring
- Targeted drug delivery
- Gene Therapy

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Courtesy: Evan Unger – ImaRx
Targeted US with labelled MB (c/o C. Caskey, PhD)

Inject microbubbles with targeting ligand

1 Minute post-injection: some free, some bound
7 minutes post-injection: only bound remain

Leong-Po, H et al. Circulation 2003;107:455-460

Neovessels with retained targeted microbubbles

Anti-angiogenesis therapy

VEGF receptors targeting

Anti-VEGF Ab

VEGF receptor

Genentech Inc
**Imaging strategy**

- VisualSonics Inc
- B-mode ultrasound
- UCA signal
- VEGFR-2 targeted UCA imaging
- Molecular sonograms
- High VEGFR2 expression
- Low VEGFR2 expression

**Targeted ultrasound contrast agents**

- Targeting lipid
- Lipid shell
- Drugs in oil layer
- Perflurocarbon gas

**Targeted UCA as a drug delivery vehicle**

- M. Tartis et al. Ultrasound in Medicine & Biology 2006

Treating brain metastasis with BBB MB Disruption + DOX

Microbubble Rx for Alzheimers
(C. Jones Ctr, U Queensland-Science Translational Med)

Rx cleared plaque-restored memory in 75% of mice w/i several wks

Conclusions

• CE-US excellent for DDx of hepatic, renal, and ovarian masses
• Potential means to assess tumor response
• Potential means to enhance specifically targeted therapies="theranostics"
• Potential use to enhance sonothrombolysis, antibodies to β-amyloid in Alzheimer’s disease

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• Bracco (Optison/Lumason)
• Pfizer Oncology
• John Bobbitt, VUMC