DETECTION OF FUNGAL INFECTIONS WITH RADIOLABELED ANTIFUNGAL AGENTS
Clinical history
Physical examination
Laboratory tests
Imaging studies

Signs and symptoms are suggestive of an infectious or a non-infectious cause
putative site of infection

microbiological cultures of body fluids and biopsies

identification of pathogens
> 85% of patients referred to hospital are febrile due to an infection

< 50% of febrile episodes can be attributed to infections

clinical manifestations of infection are subtle, non-typical, non-existent

identification of an infection at an early stage of the disease is critical for a favourable outcome
Laboratory tests

• erythrocyte sedimentation rate
• white-blood-cell count
• acute-phase proteins
• cytokines
Can nuclear medicine make an important contribution?
Current radiopharmaceuticals:

gallium-67-citrate ($^{67}$Ga)-labelled polyclonal human immunoglobulins

indium-111 ($^{111}$In)-labelled polyclonal human immunoglobulins

technetium-99m ($^{99m}$Tc)-labelled polyclonal human immunoglobulins

($^{111}$In)-labelled autologous leukocytes

($^{99m}$Tc)-labelled autologous leukocytes

($^{99m}$Tc)-labelled antigranulocyte monoclonal antibodies (or fragments thereof)

($^{99m}$Tc)-labelled chemotactic peptides

($^{99m}$Tc)-labelled interleukins
We need a radiopharmaceutical that binds to a variety of micro-organisms with little or no binding to host cells
Antimicrobial peptides often display preferential binding to micro-organisms

Radiopharmaceuticals recruited from human antimicrobial peptides can be promising candidates to discriminate infections from inflammations
### Examples of antimicrobial proteins/peptides found in nature

<table>
<thead>
<tr>
<th>Mammals</th>
<th>Insects</th>
<th>Horseshoe crabs</th>
<th>Plants</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humans</td>
<td>α-, β-Defensins</td>
<td>Thanatin</td>
<td>Tachyplesins</td>
<td>Pediocin PA-1</td>
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<tr>
<td></td>
<td>Cathelicidins</td>
<td>Drosomycin</td>
<td>Polyphemusin</td>
<td>Leucocin A UAL-187</td>
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<td>Histatins</td>
<td>Cecropins</td>
<td>Tachystatins</td>
<td>Mesentericin Y105</td>
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<td>Lactoferrin</td>
<td>Drosocin</td>
<td>Defensins</td>
<td>Sakacin P</td>
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<td></td>
<td>Ubiquicidin</td>
<td>Attacin</td>
<td>Anti-LPS factor</td>
<td>Tigerinins</td>
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<tr>
<td>Rhesus macaque</td>
<td>α-, β-, θ-defensins</td>
<td>Metchnikowin</td>
<td>Thionins</td>
<td>Ranatuerins</td>
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<td></td>
<td>Cathelicidins</td>
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<td>Defensins</td>
<td>Palustrins</td>
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<td>Pigs</td>
<td>Protegrins</td>
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<td>Shepherins</td>
<td>Ranalexins</td>
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<td></td>
<td>PR39</td>
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<td>Gaegurins</td>
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<td></td>
<td>Prophenin</td>
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<td>Nigrocin</td>
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<td></td>
<td>Cecropin</td>
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<td></td>
<td>Nisin</td>
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<tr>
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<td>Cathelicidins</td>
<td></td>
<td></td>
<td>Curvacin A</td>
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<tr>
<td></td>
<td>β-Defensin-1</td>
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<td>Nisin</td>
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<td>Frogs</td>
<td>Magainins</td>
<td></td>
<td></td>
<td>Nisin</td>
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<td></td>
<td>Dermaseptins</td>
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<td></td>
<td>Cepropin-like peptide</td>
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<td></td>
<td>Esuculentins</td>
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<td>Brevinins</td>
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<td></td>
<td>Bombinin</td>
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<td></td>
<td>Temporins</td>
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<td>Japonicins</td>
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<td>Tigerinins</td>
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<td>Gaegurins</td>
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<td></td>
<td>Nigrocin</td>
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</table>
Structural classes of antimicrobial peptides. (A) Mixed structure of human 
β-defensin-2 (PDB code 1FQQ) (216); (B) looped thanatin (PDB code 8TFV) 
(156); (C) β-sheeted polyphemusin (PDB code 1RKK) (202); (D) rabbit kidney 
defensin-1 (PDB code 1EWS) (165); (E) α-helical magainin-2 (PDB code 
2MAG) (76); (F) extended indolicidin (PDB code 1G89) (212). The disulfide 
bonds are indicated in yellow, and the illustrations have been prepared with 
use of the graphic program MolMol 2K.1 (132). 
Production of sufficient amounts of cationic peptides under GLP conditions:

- recombinant cationic-peptide production by bacteria

- peptide synthesis:
  
  chemical variants
  introduction of chelators
Radiolabeling of peptides

the radionuclide should be firmly attached to the peptide

or incorporated into the peptide

labeling should not affect the binding activity of the peptide
Selection of $^{99m}$Tc-labeled antimicrobial peptides for scintigraphic studies

- in vitro binding studies
- in vivo binding studies
- pharmacokinetics
In vitro binding studies of $^{99m}$Tc-peptides to micro-organisms and activated leukocytes

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Binding per $10^7$ cells (% of added radioactivity)</th>
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<tbody>
<tr>
<td></td>
<td>$Staphylococcus$</td>
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<tr>
<td></td>
<td>$aureus$</td>
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<tr>
<td>$^{99m}$Tc-hLF 21-31</td>
<td>1±1</td>
</tr>
<tr>
<td>$^{99m}$Tc-hLF 1-11</td>
<td>24±1</td>
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<tr>
<td>$^{99m}$Tc-UBI 18-35</td>
<td>73±14</td>
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<td>$^{99m}$Tc-UBI 31-38</td>
<td>63±16</td>
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<tr>
<td>$^{99m}$Tc-UBI 22-35</td>
<td>83±10</td>
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<td>$^{99m}$Tc-UBI 29-41</td>
<td>41±6</td>
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<tr>
<td>$^{99m}$Tc-HNP 1-3</td>
<td>48±20</td>
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<tr>
<td></td>
<td>$Klebsiella$</td>
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<tr>
<td></td>
<td>$pneumoniae$</td>
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<tr>
<td></td>
<td>$Candida$</td>
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<tr>
<td></td>
<td>$albicans$</td>
</tr>
<tr>
<td></td>
<td>$Activated$</td>
</tr>
<tr>
<td></td>
<td>$leukocytes$</td>
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<td>$^{99m}$Tc-hLF 21-31</td>
<td>2±2</td>
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<tr>
<td>$^{99m}$Tc-hLF 1-11</td>
<td>25±8</td>
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<td>$^{99m}$Tc-UBI 18-35</td>
<td>52±11</td>
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<tr>
<td>$^{99m}$Tc-UBI 31-38</td>
<td>34±13</td>
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<tr>
<td>$^{99m}$Tc-UBI 22-35</td>
<td>41±27</td>
</tr>
<tr>
<td>$^{99m}$Tc-UBI 29-41</td>
<td>15±6</td>
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<tr>
<td>$^{99m}$Tc-HNP 1-3</td>
<td>37±12</td>
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Values are means±SEM of at least four observations.


n.d. = not done
Biodistribution of $^{99m}$Tc-labeled hLF 1-11 in a normal rabbit at various time intervals.

Biodistribution of $^{99m}$Tc-UBI 29-41 in a healthy rabbit at 2 h after i.v. injection of the radiolabeled peptide.

$^{99m}$Tc-labeled compound (i.v.)

\[ \text{Scintigraphy (T/NT ratio)} \]

$\text{CFU/g tissue}$

0 18 h

\[ \text{i.m.} \]

$10^6$ CFU micro-organisms

$10^8$ heat-killed micro-organisms

50 ng LPS
$^{99m}$Tc-$\alpha$ defensins (HNP 1-3; blue bars),
$^{99m}$Tc-UBI 29-41 (yellow bars)
$^{99m}$Tc-IgG (red bars)

* $p < 0.05$ compared with the values for mice with an inflammatory process according to Student $t$ test

the ideal tracer for infection imaging should fulfill the following criteria:

i) rapid uptake at sites of infection with little or no accumulation at sites of sterile inflammation;

ii) good stability of the labeled complex under physiological conditions;

iii) preservation of binding activity upon labeling;

iv) rapid clearance from the circulation with little or no accumulation in unaffected tissues,

v) little or no adverse effects, such as toxicity and immunological reactions
two new groups of tracers have been introduced

$^{99m}$Tc-labeled ciprofloxacin ($^{99m}$Tc-Infecton)

$^{99m}$Tc-labeled fluconazole
Can $^{99m}$Tc-labeled antimicrobial peptides, $^{99m}$Tc-fluconazole visualize *C. albicans* infections?
fluconazole-resistant *C. albicans*-infected mice

C. albicans-infected mice

$^{99m}$Tc-Fluconazole

$^{99m}$Tc-UBI 29-41

$^{99m}$Tc-hLF(1-11)

$^{99m}$Tc-Fluconazole in LPS inflamed thigh muscle

Right: scintigraphic imaging of the biodistribution of the tracers in the entire animal.

Left: scintigrams of the same animal with higher contrast visualising the thigh muscle infection/inflammation indicated by an arrow at 1 h after injection of the tracers.

\textit{C. albicans}-infected mice (open bars) mice inflamed with heat-killed \textit{C. albicans} (hatched bars) or lipopolysaccharide (LPS, closed bars)

$^{99m}\text{Tc}$-labeled fluconazole

![Graph showing the relationship between T/NT ratio and log CFU/g tissue for C. albicans. The graph has a line of best fit with a $R^2 = 0.864, P < 0.05$.]

Can nuclear medicine contribute in monitoring the efficacy of antifungal therapy?
Monitoring the efficacy of antifungal therapy by accumulation of $^{99m}$Tc-UBI 29-41
Is $^{99m}$Tc-fluconazole able to discriminate between *C. albicans* and bacterial infections?
Mice infected/inflamed with:
- C. albicans (open bars)
- MRSA (diagonally hatched bars)
- K. pneumoniae (vertically hatched bars)
- Heat-killed C. albicans (dotted bars)
- LPS (closed bars)

**99mTc-fluconazole**

**99mTc-IgG**

*C. albicans*
Are $^{99}$mTc-antimicrobial peptides able to discriminate between *C. albicans* and bacterial infections?
Mice infected/inflamed with:
- *C. albicans* (open bars)
- MRSA (diagonally hatched bars)
- *K. pneumoniae* (horizontally hatched bars)
- heat-killed *C. albicans* (dotted bars)
- LPS (closed bars)

**99mTc-hLF 1-11**

**99mTc-UBI 18-35**

**99mTc-UBI 29-41**

*C. albicans* infected mice
Can $^{99m}$Tc-labeled antimicrobial compounds visualize *A. fumigatus* infections?
cyclophosphamide (i.p.) 99mTc-labeled compound (i.v.)

\[ \downarrow \quad \downarrow \quad \downarrow \]

\[ \text{Scintigraphy (T/NT ratio)} \quad \text{CFU/g tissue} \]

-72 h 0 18 h

\[ \text{i.m.} \]

\[ 10^4 \text{ Aspergillus fumigatus conidia} \]

\[ 10^8 \text{ heat-killed micro-organisms} \]

\[ 50 \text{ ng LPS} \]
**A. fumigatus infected leukocytopenic mice**

- **$^{99mTc}$-fluconazole (A)**
- **$^{99mTc}$-hLF 1-11 (B)**
- **$^{99mTc}$-UBI 29-41 (C)**
- **$^{99mTc}$-IgG (D)**

**A. fumigatus (open bars)**
**LPS (closed bars)**

A. fumigatus infected leukocytopenic mice (open bars) or inflamed with LPS (closed bars)
A. *fumigatus* infected leukocytopenic mice (open bars) or inflamed with LPS (closed bars)

$^{99m}$Tc-hLF 1-11  $^{99m}$Tc-UBI 18-35  $^{99m}$Tc-UBI 29-41
Biodistribution of $^{99m}$Tc-labelled compounds in mice infected with *C. albicans*

<table>
<thead>
<tr>
<th>$^{99m}$Tc compound</th>
<th>Injected radioactivity (% injected dose)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Bladder 15 min</td>
<td>60 min</td>
<td>240 min</td>
<td>Kidneys 15 min</td>
<td>60 min</td>
<td>240 min</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>29±3</td>
<td>34±2</td>
<td>29±7</td>
<td>24±2</td>
<td>22±7</td>
<td>22±4</td>
</tr>
<tr>
<td>hLF 1-11</td>
<td>12±2</td>
<td>18±3</td>
<td>27±3</td>
<td>15±3</td>
<td>15±2</td>
<td>19±2</td>
</tr>
<tr>
<td>UBI 29-41</td>
<td>23±3</td>
<td>32±5</td>
<td>17±3</td>
<td>19±2</td>
<td>22±2</td>
<td>12±2</td>
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<td>IgG</td>
<td>17±3</td>
<td>47±2</td>
<td>7±3</td>
<td>14±7</td>
<td>20±2</td>
<td>18±2</td>
</tr>
</tbody>
</table>

Values are the mean±SD of at least four observations

Accumulation of $^{99m}$Tc-labeled hLF 1-11 in MRSA-infected thigh muscles in mice at various intervals after different routes of administration: iv. (open bars) ip. (closed bars) subcutaneous (grey bars) oral (hatched bars) Results are the means ± S.E.M. of at least eight animals.

... and in clinical studies?
(A) Patient with a negative $^{99m}$Tc-UBI 29-41 scintigraphy. (B) Patient with a positive $^{99m}$Tc-UBI 29-41 scintigraphy. Dose injected 740 MBq (20 mCi), 500 kilocounts (kcts) per scan. MCts, mediastinum counts; LCts, lung counts; M/L ratio, mediastinum/lung counts ratio.
