EVALUATION SUR MODELE MURIN DES OSTEOSARCOMES PAR IRM BOLD ET CORRELATION A L’IMAGERIE DE DIFFUSION ET AU TEP-TDM F-MISO : ETUDE PRELIMINAIRE

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Introduction:

- Tumors oxygen concentration: prognostic and therapeutic implications in many solid tumors. (9,10,7,11,18,16)

- Calcul of tissue oxygenation not performed in practice.

- BOLD MRI objective: non invasive evaluation of tissue oxygenation (8)
**Technical Recalls:**

- **BOLD (Blood Oxygenation Level Dependent):** principle of endogenous contrast (12,3,6)
- Paramagnetic Effect of deoxy-Hb
- Increase of deoxy-Hb accelerates relaxation time of $T_2$

- Tissues with high $O_2$ consumption produce more deoxy-Hb:
  - increase of BOLD signal intensity (2,17)
Epidemiologic Reminders:

- Osteosarcomas: Most frequent primitive malignant bone tumors (30%) (4)
- Hypoxic PET-CT f-MISO (1)
- Affect metaphyseal +++ (femur and humerus)
- MRI is the gold standard (4) for study of:
  - tumor’s upward extension
  - transphyseal extension
  - «skip metastases»
  - soft parts study
PURPOSE:

- Evaluate hypoxic character of osteosarcoma on murin model with BOLD MRI.
- Correlation with ADC Cartography, PET-CT f-MISO and specific anatomopathology oxygenation study.
- Interest of BOLD MRI in Musculo-Squeletal oncology?
Material and Method:

• Animal and Tumoral model:
  • 8 male rats Sprague-Dawley
  • Immunocompetents
  • Transplantation of osteosarcoma on right femur (Allouche model 1980 after radioactive cerium bone injection)

• Model of hypoxia:
  • Ligation of the femoral artery in 2 non-grafted rats
  • Comparative analysis of two lower limbs with pediatric pulse oximetry.
Material and Method:

• Anesthesia:
  • Isoflurane® (4% for induction and 2%)
  • Ketamine® (5mg/kg) – Largactil® (1.5 mg / kg)(5)

• MRI:
  • MRI Philips® Intera 3 Tesla
    • Neurovascular Antenna
    • Rats placed in prone position, head maintained in the mask of O₂.
    • Monitoring: heart rate and pulse oximetry.

• 3 MRI D10, D17 and D24 after tumoral induction.
Material and Method:

- **Sequences:**
  - Survey
  - Axial T1 FSE
  - Axial T2 FSE
  - Axial diffusion EPI + ADC map, b0 and b1000
  - Axial BOLD on ambient air, and after O₂ impregnation (2 and 3 min)
Survey of the rat, prone position, back legs to the front, in neurovascular antenna
Sequence Axial T1, without fat sat
Right femoral osteosarcoma in Hyposignal T1

Sequence Axial T2, without fat sat
Right femoral osteosarcoma in Hypersignal T2
Sequence Axial Diffusion, inverse mode PET like
Right femoral osteosarcoma with Hypersignal Diffusion

ADC Map
Right femoral osteosarcoma with Diffusion Restriction
Right femoral osteosarcoma
BOLD Sequence in ambient air
Material and Method:

• Measures:
  - Philips View Forum Console 4.3
  - Program “BOLD MRI "Philips

• Anatomical identification, volumic calcul and identification of potential areas of necrosis on sequence T1 and T2.
* **Measures:**

  * Measures (in absolute signal) on sequences BOLD and Diffusion on healthy tissue, tumor and fat-free without $O_2$ and after $O_2$ impregnation at 2 and 3 minutes.

  * Comparative study of 2 ROI (min 4 pixels) on 2 different slices by 2 independent operators.

  * Comparison of the tumor in AA, and after impregnation at 2 and 3 minutes.

  * Comparison between normal tissue and tumor in AA and after impregnation at 2 and 3 minutes.
Ligation of the right femoral artery
ROI in the muscles of the right thighbone hypoxic (red) and left in non hypoxic (pink)
Material and Method:

- **PET CT-fMISO:**
  - Siemens unit, 8 bars.
  - Review conducted between J24 and J26.
  - Injection of 1.5 mg / kg f-MISO then 40min after purchase.
  - Identical position on MRI.
  - Sedation with isoflurane.
  - Measuring areas of interest of tumor area and contralateral normal tissue on 2 different cuts by 2 independent operators.
  - Measuring the ratio: tumor tissue / Healthy tissue (normoxia < 1; Hypoxia > 1.2)
PET CT with right osteosarcoma.
Material and Method:

- **Anatomo-Pathology:**
  - Osteoblastic tumor cells develop an atypical immature bone or osteoid.
  - Mitotic activity +++, abnormal mitosis
  - Analysis of all rats after the study MRI and PET Search specific tissue hypoxia.

- Hypoxia (hif1 in green)
Material and Method:

• **Statistical Methodology:**
  
  * Quantitative variables represented in the forms of average, standard deviation and median.
  
  * Comparison by non-parametric paired test of Wilcoxon.
  
  * Comparison of three variables by non-parametric paired test of Friedman.
  
  * Correlations between tumor volume and differences of signal were made by estimating Spearman correlation coefficients.
  
  * $p < 0.05$. 
Material and Method:

- Are Osteosarcomas known to be hypoxic assessed by technique of BOLD MRI?

- Is there a significant difference in signal, marking hypoxia in tumor tissue between ambient air and after impregnation at 2 then 3 minutes?

- Is there a significant difference in signal marking hypoxia between tumor tissue and contralateral normal tissue in ambient air, and after impregnation at 2 then 3 minutes?
Material and Method:

- Is there a correlation between hypoxia evaluated by BOLD and ADC ratio?

- Is there a correlation between hypoxia evaluated by BOLD and Maximum SUV in PET–CT?
# Results:

* Tumoral Study:

<table>
<thead>
<tr>
<th></th>
<th>J10</th>
<th>J17</th>
<th>J24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumoral volume</td>
<td>0,1 cm³</td>
<td>1,1 cm³</td>
<td>3,5 cm³</td>
</tr>
<tr>
<td>AA signal</td>
<td>529</td>
<td>597</td>
<td>625</td>
</tr>
<tr>
<td>Signal after 2min O₂</td>
<td>290</td>
<td>478</td>
<td>487</td>
</tr>
<tr>
<td>Signal after 3min O₂</td>
<td>394</td>
<td>471</td>
<td>482</td>
</tr>
<tr>
<td>ADC coefficient</td>
<td>0,66</td>
<td>0,57</td>
<td>0,44</td>
</tr>
</tbody>
</table>
Are Osteosarcomas known to be hypoxic by the technique of BOLD MRI?

Is there a significant difference in signal marking hypoxia in tumor tissue between ambient air and after impregnation at 2 and then 3 minutes?

- **Yes:** significant difference between AA and the tumor under O2 at 2 and 3 minutes ($p = 0.018$)

- **No** significant difference between the signal between 2 and 3 minutes
Results:

* Are Osteosarcomas known to be hypoxic assess by technique of BOLD MRI?

* Is there a significant difference in signal marking hypoxia between tumor tissue and contralateral normal tissue in ambient air, and after impregnation at 2 then 3 minutes?

* Yes, significant difference between the tumor and healthy tissue in AA and after impregnation at 2 then 3 minutes (p = 0.018 to D17 and D24)
Results:

• Is there a correlation between hypoxia evaluated by BOLD and ADC ratio?

  • **No** correlation between difference in signal AA at 2 and 3 minutes of oxygenation for the tumor.

  • **No** correlation between signal difference between normal tissue and tumor.
Results:

- Is there a correlation between hypoxia evaluated by BOLD and PET CT Max SUV?

  - Yes: 100% correlation
Discussion:

- Significant difference in signal between the tumor and healthy tissue.
- Significant difference in tumor signal in AA, and at 2 and 3 min of impregnating $O_2$.
- Not effect the volume or the time of impregnation.
- Superposable results of BOLD and PET-CT fMISO (qualitative? Quantitative? and anatomic?)
Discussion:

• **Interests:**
  - Diagnosis (nature of the hypoxic tumor)
  - Therapeutic (mainly radiotherapy)

• **Prognostic:**
  - independant factor of the TNM

• **Bias:**
  - Heterogeneous BOLD response
  - Tissues contamination by T2 effect (no T1 calcul before gadolinium in practice)
  - 3T MRI Non research dedicated.
Conclusion:

- **BOLD MRI:**
  - Legitimates her place in oncological imaging.
  - Promising tool in non-invasive functional imaging (non irradiant)
  - But need further development for use in clinical practice.
  - Low anatomical resolution (vs PET)
Bibliography:


12) Ogawa S., Lee T.M., Kay A.R, Tank D.W., Brain magnetic resonance imaging with contrast dependent on blood oxygenation, proceedings of the National Academy of Sciences of the USA, 1990, 87:9868-9872.


