Very late effects of adjuvant chemotherapy on the brain

V Koppelmans; MB de Ruiter; M de Groot; F van der Lijn; H Vrooman; W Boogerd; C Seynaeve; WJ Niessen; MW Vernooij; A van der Lugt; MMB Breteler; SB Schagen
Introduction: Background

- Adjuvant chemotherapy has been associated with cognitive problems, brain volume reductions and lower white matter integrity up to several years after treatment. Wefel et al., 2011 Lancet Oncol. Inagaki et al., 2007, Cancer; McDonald et al., 2010, Breast Cancer Res Treat; Abraham et al., 2008, Clin Breast Cancer; Deprez et al., 2010, Hum Brain Map; Deprez et al., 2012, JCO

- A limited number of studies has associated adjuvant chemotherapy with very late cognitive problems and brain changes de Ruiter et al., 2011a&b, Hum Brain Map; Yamada et al., 2010 J Neuropsychiatry Clin Neurosci; Koppelmans et al., 2012, JCO

- Insight into the long-term effects of chemotherapy on the brain, as well as underlying mechanisms is still limited Schagen et al., 2007, Lancet Oncol; Vardy et al., JCO, 2007; Ahles et al., Nat Rev Cancer, 2007; Wefel et al., Cancer, 2010
Introduction: Research question

Is adjuvant chemotherapy for breast cancer associated with cognitive functioning* and brain morphology at very long time after cessation of treatment?

*Koppelmans et al., 2012, JCO
Methods: Study population

- female breast cancer survivors treated with radiotherapy
- treated in either of the two Dutch specialized cancer hospitals

Eligibility criteria:
- between 50 and 80 years of age
- treatment received between 1976-1995
- disease free survival since primary breast cancer
- treated with 6 cycles of CMF chemotherapy:
  - Cyclophosphamide Methotrexate 5-Fluorouracil
- no adjuvant endocrine treatment
Methods: the Rotterdam Study

• Prospective population based cohort study

• 15,000 subjects aged 45+ who entered the study since 1990

• Follow-up every 3 to 4 years

• Various outcomes:
  – Cardiovascular
  – Neurologic
  – Endocrine
  – Ophthalmologic

Hofman, Breteler et al., The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol. 2011
Ikram, Breteler et al., The Rotterdam Scan Study: design and update to 2012. Eur J Epidemiol. 2011
Methods: Reference population

- Female participants of the Rotterdam Study
- Age matched (1:2)
- Without a history of cancer
- Sample comparable, but not identical for different analyses
### Methods: MRI examination

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>MRI sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volumetrics:</strong></td>
<td>T1</td>
</tr>
<tr>
<td>total brain tissue volume (gray &amp; white matter)</td>
<td></td>
</tr>
<tr>
<td>hippocampal volume</td>
<td></td>
</tr>
<tr>
<td>focal gray matter: ‘voxel based morphometry’</td>
<td></td>
</tr>
</tbody>
</table>

Scanner: General Electric 1.5 Tesla
Methods: Participation Rate

- **Eligible**: 
  - N = 292

- **Decliners**: 
  - N = 96 (32.9%)

- **Participants**: 
  - N = 196 (67.1%)

- **Completed MRI**: 
  - N = 191
### Results: Population Characteristics & Confounders (1/2)

<table>
<thead>
<tr>
<th></th>
<th>Chemo survivors</th>
<th></th>
<th>Reference group</th>
<th></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Sd</td>
<td>Mean</td>
<td>Sd</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.1</td>
<td>6.5</td>
<td>64.1</td>
<td>6.4</td>
<td>.99</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.1</td>
<td>6.2</td>
<td>162.8</td>
<td>6.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.1</td>
<td>13.3</td>
<td>72.8</td>
<td>11.7</td>
<td>.81</td>
</tr>
</tbody>
</table>
### Results: Population Characteristics & Confounders (2/2)

<table>
<thead>
<tr>
<th></th>
<th>Chemo survivors</th>
<th>Reference group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Anti-hypertensive Medication</td>
<td>66</td>
<td>35.3</td>
<td>117</td>
</tr>
<tr>
<td>Cholesterol-lowering Medication</td>
<td>34</td>
<td>18.2</td>
<td>89</td>
</tr>
<tr>
<td>Diabetes</td>
<td>13</td>
<td>7.0</td>
<td>14</td>
</tr>
</tbody>
</table>
total tissue volumes

- SPM8 was used for segmentation: ‘new segment’
- based on tissue probability maps and voxel intensity
- volumes: gray & white matter, cerebrospinal fluid, total brain volume
### MRI OUTCOME MEASURE: TOTAL TISSUE VOLUMES

<table>
<thead>
<tr>
<th>MRI OUTCOME MEASURE:</th>
<th>Chemo survivors (n=184)</th>
<th>Reference group (n=368)</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (ml)</td>
<td>sd</td>
<td>Mean (ml)</td>
</tr>
<tr>
<td>Intracranial volume</td>
<td>1319</td>
<td>137</td>
<td>1316</td>
</tr>
<tr>
<td>Brain volume</td>
<td>1087</td>
<td>24</td>
<td>1091</td>
</tr>
</tbody>
</table>

Comparable to decline in gray matter volumes of ~4 years of age

Within the chemotherapy-exposed survivors increasing ‘time since treatment’ was associated with smaller gray matter volume

*Koppelmans et al., 2012, Breast Cancer Res Treat*
hippocampal volume

Coronal slices of graph-cut-based automated segmentation of the hippocampus *van der Lijn et al., 2008, NeuroImage*
### MRI Outcome Measure: Hippocampal Volume

<table>
<thead>
<tr>
<th></th>
<th>Chemo Survivors (n=184)</th>
<th>Reference Group (n=368)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (ml)</td>
<td>sd</td>
<td>Mean (ml)</td>
</tr>
<tr>
<td>Left hippocampus</td>
<td>2.874</td>
<td>0.40</td>
<td>2.920</td>
</tr>
<tr>
<td>Right hippocampus</td>
<td>2.868</td>
<td>0.40</td>
<td>2.874</td>
</tr>
</tbody>
</table>

*Koppelmans et al., 2012, Breast Cancer Res Treat*
focal gray matter: Voxel

- Voxel Based Morphometry under SPM8 (DARTEL)
- normalization: register each brain to a study-specific template
- modulation: keep information of original volumes
- **No differences in focal gray matter volume**
white matter microstructural integrity

- focal: *tract based spatial statistics* (TBSS under FSL)
- create study specific white matter skeleton
- global: mean DTI parameters throughout
  a) the normal appearing white matter
Association between chemotherapy and Fractional Anisotropy

- No differences in **focal** white matter quality between groups (TBSS)

- No differences in **global** (mean) white matter quality between groups
Association between time since treatment and **Fractional Anisotropy**

FA (white matter integrity) decreases with longer time since treatment
Association of time since treatment with white matter integrity

<table>
<thead>
<tr>
<th>Measure</th>
<th>95% CI for $\beta$</th>
<th>Lower</th>
<th>Upper</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Fractional Anisotropy in:</td>
<td>$\beta$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>the Normal Appearing White Matter</td>
<td>$-4.6 \times 10^{-4}$</td>
<td>$-8.1 \times 10^{-4}$</td>
<td>$-1.1 \times 10^{-4}$</td>
<td>.011</td>
</tr>
</tbody>
</table>

Decrease in Fractional Anisotropy per year ‘time since ‘treatment’
cerebral blood flow & cerebral perfusion

A sagittal angiographic scout image for localization of the phase-contrast imaging plane (red line) perpendicular to the carotid and basilar arteries
Association of chemotherapy with cerebral blood flow and perfusion

<table>
<thead>
<tr>
<th>MRI OUTCOME MEASURE:</th>
<th>CEREBRAL BLOOD FLOW &amp; PERFUSION</th>
<th>RESULTS</th>
</tr>
</thead>
</table>

Chemotherapy-exposed breast cancer survivors (n=187) | Reference group (n=374) | 95% CI for $\beta$ |

<table>
<thead>
<tr>
<th></th>
<th>Mean (Sd)</th>
<th>Mean (Sd)</th>
<th>$\beta$</th>
<th>Lower</th>
<th>Upper</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>tCBF</td>
<td>515.5 (91.2)</td>
<td>518.1 (90.7)</td>
<td>-2.6</td>
<td>-18.7</td>
<td>13.6</td>
<td>.75</td>
</tr>
<tr>
<td>perfusion</td>
<td>57.8 (9.4)</td>
<td>58.2 (9.3)</td>
<td>-0.5</td>
<td>-2.1</td>
<td>1.2</td>
<td>.58</td>
</tr>
</tbody>
</table>

$tCBF = \text{total cerebral blood flow (ml/min)}$

$\text{perfusion = cerebral blood flow per 100ml brain}$

within the 187 survivors, ‘time since treatment’ was not associated with total cerebral blood flow or perfusion
Discussion: summary

More than 20 years post-treatment, adjuvant CMF chemotherapy for breast cancer is...

...associated with:

- smaller global gray matter volume

Furthermore: with *increasing* time since treatment white matter integrity and gray matter volume *decreases* independently of age
Discussion: interpretation

• The observed smaller gray matter volume in chemotherapy-exposed patients might (partially) explain the observed worse cognitive functioning in this group of patients Koppelmans et al., 2012, JCO

• No indication that the observed smaller gray matter volume in this group of patients results from chemotherapy induced changes in cerebral blood flow

• Association between time since treatment and white matter integrity calls for prospective studies on the very late effects of chemotherapy
Discussion: limitations

- Cross-sectional design (no baseline measurement)
- Unable to distinguish the difference between the effect of chemotherapy, breast cancer or both
- CMF is not the main choice of adjuvant treatment nowadays
Sanne Schagen, PhD
NKI-AvL dept. of Psychosocial Research

Monique Breteler, MD, PhD
ErasmusMC dept. of Epidemiology

Willem Boogerd, MD, PhD
NKI-AvL dept. of Neuro-Oncology

Aad van der Lugt, MD, PhD
ErasmusMC dept. of Radiology

Caroline Seynaeve, MD, PhD
ErasmusMC – Daniel den Hoed Clinic

Michiel de Ruiter, PhD
NKI-AvL dept. of Psychosocial Research
Relevance

If CMF chemotherapy is associated with brain structural changes:

1. Patient attention and concerns which will amplify the request for information and treatment interventions

2. Need for additional research regarding the late effects and clinical implications of contemporary cytostatics
Introduction: Candidate mechanisms

- Direct neurotoxic injury
- Microvascular injury
- Immunologic-driven processes
- Decreased neurogenesis
- Neurochemical changes
- Hormonal-driven processes

Not mutually exclusive & many factors involved
- Treatment regimens, dosimetry, sequence and timing
- Host individual differences
Cohorts of the Rotterdam Study

First cohort:
- RS-I-1: n = 7983
  07/1989-09/1993
- RS-I-2: n = 6315
  09/1993-12/1995
- RS-I-3: n = 4797
  03/1997-12/1999
- RS-I-4: n = 3850
  01/2002-07/2004
- RS-I-5: n = ??
  03/2009-??

Second cohort:
- RS-II-1: n = 3011
  02/2000-12/2001
- RS-II-2: n = 2389
  07/2004-12/2005
- RS-II-3: n = ??
  ??-??

Third cohort:
- RS-III-1: n = 3932
  02/2006-12/2008
- RS-III-2: n = ??
  ??-??
MR outcome measures:

white matter lesion volume
MR outcome measures:

cerebral microbleeds
Introduction: Evidence from structural MR studies:

9 studies up to maximum 3.5 years after treatment
1 study by de Ruiter et al. 10 years post treatment

- more diffuse **white matter** lesions
- white matter pathology may be partially transient
- **10 years after treatment**: lower white matter quality of major fiber tracts
  
  *Brown et al., Am J Neuroradiol* 1995; *Ferguson et al., JCO* 2007; *Deprez et al., Hum Brain Map* 2010; *de Ruiter et al., Hum Brain Map* 2011b

- **gray matter** differences less consistent
  
  - local differences 3-4 months after chemotherapy
  - partial recovery seen one year from baseline
  - **10 years after treatment**: focal gray matter volume reductions

  *Inagaki et al., Cancer 2007, MacDonald et al., Breast Cancer Res Treat* 2010
Introduction: Animal studies (1)

Chemotherapy affects brain cells:

- Cytotoxic agents (e.g. cyclophosphamide) have been associated with memory impairment in rodents at clinically relevant doses.
- Methotrexate decreases hippocampal cell proliferation in rats (Seigers et al. 2009).

5-FU damages white matter tracts and is toxic for progenitor cells and non-dividing oligodendrocytes.

Chemotherapy also affects the cerebro-vascular system:

- Methotrexate (MTX): reduction in hippocampal blood vessel density (rats)
- MTX and cyclophosphamide: endothelial cell damage (mice)
- MTX: apoptosis in post-mitotic endothelial cells (bovine cell cultures)
Introduction: Evidence from functional MR studies:

• Four studies

• Chemotherapy exposed patients showed lower activation during cognitive task performance in task associated brain areas compared to healthy/cancer controls

• Hypoactivation is associated with worse task performance

Saykin et al., (conference abstract) 2006; Ferguson et al., JCO, 2007; Kessler et al., Clin Cancer Res, 2009; de Ruiter et al., Hum Brain Map 2011a
Methods: Examination

• Rotterdam Study (RS) research center visit in the Ommoord district, Rotterdam

• abbreviated RS examination (~3 hours):
  – Blood draw and height & weight measurement
  – Carotid artery ultrasound + electrocardiogram
  – Interview
  – Neuropsychological examination
  – MRI examination
Methods: voxel

Voxel = 3D pixel
Methods: Analyses

Covariates:
Age + Age$^2$
Height
Blood pressure
Diabetes Smoker status
Education level
Depressive symptoms CESD
White matter volume
WM lesion volume
MR outcome measures:

local gray matter differences

Voxel Based Morphometry (VBM):

• Hypothesis-free technique
• Normalization: Register each brain to a template
• Modulation: keep information of original volumes
• Smoothed so that each voxel represents the average of itself and its neighbors
• Comparison (GLM) at the voxel level
MR outcome measures:
hippocampal volume
MR outcome measures:
microstructural white matter integrity

Diffusion in 3-D: Homogeneous Medium

Water in a Homogeneous Medium
Water Motion
Diffusion [Sphere]
MR outcome measures:
microstructural white matter integrity

Diffusion in 3-D: White Matter

Water in an Oriented Tissue

Water Motion

Ellipse

Borrowed from Wang Zhan, PhD, UCSF
MR outcome measures:

Microstructural white matter integrity

DTI: several diffusivity measures

Axial diffusivity ($\lambda_\parallel$) = $\lambda_1$

~axonal damage

Radial diffusivity ($\lambda_\perp$) = ($\lambda_2 + \lambda_3$)/2

~myelin damage

Mean diffusivity (MD) = ($\lambda_1 + \lambda_2 + \lambda_3$)/3

MR outcome measures: microstructural white matter integrity

Diffusion in white and gray matter

White matter pixel

Gray matter pixel:
healthy white matter

mean diffusion shape

impaired white matter

mean diffusion shape

direction of diffusion is described by *Fractional Anisotropy* (ranges from 0 to 1)
Diffusion Tensor Imaging (DTI) allows us to measure the preferred direction of diffusion.

The non-uniformity of diffusion with direction is usually described by the term Fractional Anisotropy (FA).
Results: TBSS

- Model 1: age
- Model 2: all

Analyses:
- cancer survivors vs. reference group
- effect of time since treatment (within survivors)
Red-yellow color: lower FA

Black lines: mean skeleton
Results: TBSS: Group comparison

Model 1

Model 2
Results: Whole brain DTI parameters

Mean Fractional Anisotropy throughout the White Matter Skeleton

<table>
<thead>
<tr>
<th></th>
<th>Chemo survivors (n=184)</th>
<th>Reference group (n=368)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
<td>Mean</td>
</tr>
<tr>
<td>Mean Fractional Anisotropy</td>
<td>0.435</td>
<td>0.014</td>
<td>0.434</td>
</tr>
</tbody>
</table>

Mean Fractional Anisotropy in the Normal Appearing White Matter

<table>
<thead>
<tr>
<th></th>
<th>Chemo survivors (n=184)</th>
<th>Reference group (n=368)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
<td>Mean</td>
</tr>
</tbody>
</table>
Discussion: Voxel Based Morphometry

• de Ruiter et al., *Hum Brain Map, in press*
  17 chemo$^+$ versus 15 chemo$^-$
  ~10 years post treatment
  focal gray matter reductions in posterior parts of the brain

• McDonald et al., *Breast Cancer Res Treat, 2010*
  17 chemo$^+$ vs. 12 chemo$^-$ vs. 18 healthy controls
  0, 1, 12 months post treatment
  - decline in gray matter from baseline to 1 month
  - recovery at 12 months in some but not all regions
Discussion: Hippocampal volume

• Yoshikawa et al., *Breast Cancer Res Treat*, 2005
  44 chemo+ versus 31 chemo-
  3.5 years post treatment
  no differences in hippocampal volume

• Seigers et al., *Behav Brain Res*, 2009
  Rats injected with high-dose methotrexate
  1 day – 3 weeks post chemotherapy
  decreased hippocampal cell proliferation, associated with memory dysfunction
Discussion: TBSS (1)

- **Abraham et al., Clin Breast Canc, 2008**
  10 chemo+ versus 9 healthy controls
  1.8 years post treatment
  lower FA in the genu of the corpus callosum

- **de Ruiter et al., Hum Brain Map, in press**
  17 chemo+ versus 15 chemo-
  ~10 years post treatment
  widespread reductions in white matter quality including the corpus callosum
Discussion: TBSS (2)

• Deprez et al., *Hum Brain Map*, 2010
  17 chemo\(^+\) vs. 10 chemo\(^-\) vs. 18 healthy controls
  3 months post treatment
  chemo\(^+\) lower white matter quality in frontal
  & temporal regions