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CONTRACTING ORGANIZATION: Boston University School of Medicine

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14. ABSTRACT

Gulf War Illness (GWI) represents a cluster of multi-system chronic symptoms experienced by a third of veterans who served in the Gulf War. To date, accurate detection, and evaluation of ongoing pathology in GWI remains challenging due to the highly heterogeneous symptom profiles within cases and the lack of validated biological tests/markers of the illness. Accurate identification of individuals at earlier stages of the disease is vital to providing therapeutic interventions when these are likely to be most effective to improve the quality of life of veterans with GWI. The research team have found novel imaging features from multi-modal magnetic resonance imaging (MRI) in their previous works. This project is designed to expand our work to a larger multi-site database, the Boston Biorepository, Recruitment, and Integrative Network (BBRAIN), to validate neuroimaging markers further and identifying optimal biomarkers and computational models for diagnosis, subtyping, and informing potential determinants of GWI.

15. SUBJECT TERMS

Gulf War Illness, Mild Traumatic Brain Injury, AD, Neuroimaging, Immune markers, Cognitive markers, Machine learning

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TABLE OF CONTENTS

Introduction

Symptoms of GWI typically include persistent headaches, widespread pain, fatigue, memory and concentration problems, mood complaints, and other difficulties. The exact cause of GWI still remained unclear many years following the event, and efforts directed towards developing effective treatments for GWI have been hampered by the lack of reliable, objective biomarkers for the illness. It was not until recently when several studies suggested that potentially hazardous exposures the troops experienced during the deployment may have exerted alterations in the brain. The observed heterogeneity of symptom profiles may be a consequence of the different stressors and exposure factors (e.g., use of skin pesticide, had mild traumatic brain injury during war, etc.) eliciting distinct responses of the human immune system that interacted with the brain, suggesting a strong brain-immune component to the disorder. Recently, Dr. Koo, the principal investigator (PI) of this project, confirmed that the characterization of brain tissue microstructure and connectivity using complex diffusion magnetic resonance imaging (MRI) could provide key information for classifying GWI (GW160032, PI:Koo). PI and his team also found that the imaging profiles may differ between GW veterans and reflect complex biological changes that interact with different exposure conditions during the Gulf War (GW) and/or subject-specific persistent symptom profiles.

While the findings listed above represented important progress for understanding GWI, additional validation steps need to take place before applying suggested biomarkers to clinical settings. Recent systematic review of existing GWI biomarkers studies suggests that while most studies indicated a significant association between biological measures and risks of GWI, these findings were difficult to interpret due to the inconsistency across studies and the small control sample size. In this project, we will be able to address these issues using multimodal MRI scans obtained from a larger GWI database, the Boston Biorepository, Recruitment, and Integrative Network (BBRAIN), but necessary data harmonization steps need to be first performed on this multisite database to account for technical biases (e.g. MRI scanner, site differences) to produce generalized data elements. Based on the multisite data, we will further validate neuroimaging markers to address the following issues: 'how reliable these markers are', 'how does it relate to other biological changes' (often important for studying complex symptoms), 'how sensitive for detecting different profiles or stages', and 'whether the marker can predict further deteriorations. Successful outcome of this project will not only help us understand the complex interactions of the brain and immune system underlying GWI pathology, but will also serve as a basis for the development of a personalized medicine approach to treating this debilitating disorder. Moreover, the generalized imaging data, which will be added into BBRAIN from this project, can be used by future investigators to generate more valuable findings from the same database.

Keywords

Gulf War Illness
Toxicant exposure
Mild TBI
White matter integrity
Gray matter microstructure
Connectivity
Morphometry
Neuroinflammation
MRI marker
Machine Learning
Brain mapping

Accomplishments

- **What were the major goals of the project?**

Major goals and accomplishments for year 1 are summarized in the table below:

Specific Aim 1: Harmonization of multi-site data and testing of MRI markers for GWI.	**Timeline**	**Site 1**	**Outcomes and Reactions**
Major Task 1: Obtain IRB approval	Months		
Subtask 1: Obtain local IRB approval	1-3	Drs. Koo and Sullivan	Achieved
Subtask 2: Obtain HRPO approval	3-4	Drs. Koo and Sullivan	*Achieved*
Major Task 2: MRI image processing			
Subtask 1: Morphometry data processing – Freesurfer (including hippocampal subfields processing), morphological network processing will be performed on Alabama, Georgetown, and UCSF data (370 subjects). BU site data on this part is already processed.	4-12	Dr. Koo, Dr. Sullivan– managing data transfer from BBRAIN	*Achieved*
Subtask 2: Diffusion data processing –Low-B value GQI, and DTI processing from in-house developed pipeline (processing on Dataset-M and Dataset-S/D). Multi-B diffusion processing on Dataset-M is already processed from PI's work.	4-12	Dr. Koo	*On going: BBRAIN 2nd t.p. data, Georgetown data*
Subtask 3: Resting fmri processing- 17 resting state networks (RSNs) will be extracted from in-house developed HCP style pipeline (processing on Dataset-S/D and Dataset-M).	4-12	Dr. Koo	*Pipeline developed. Processing on-going on Georgetown data*
Subtask 4: Quality assurance processing. Both automated and manual quality assurance process will be performed on morphometry, diffusion and resting fmri processing results.	9-12	Dr. Koo (discussing with Dr. Baraniuk)	*On-going*
Major Task 3: Data Harmonization			
Subtask 1: Embedding of data harmonization framework into the computational server. In-house developed script will be optimized for BBRAIN data, installed and tested using Dataset-M (contains site1 and site2 data).	9-12	Dr. Koo	*Harmonization script built.*
Major Task 6: HCP data processing and Brain age modeling			
Subtask 1: Apply the image processing framework listed in major task 2 in HCP data.	1-15	Dr. Koo	*HCP morphometry processing done. UKbiobank data newly added to the project for normative brain model analysis.*

- **What was accomplished under these goals?**

  1) Major activities:

Major activities in this project year include followings:

Major activities in this project year include the followings: 1) Obtaining IRB/HRPO, 2) setting up on the computing environment setup; 3) building process pipelines for the neuroimaging data; 4) post-processing of neuroimaging data; 5) Quality assurance work on the processed results; 6) Processing of non-veteran MRI data for performing large-scale analysis.

- A parallel computing environment for processing neuroimaging data was built for this project. We used cluster computing system housed in Massachusetts Green High Performance Computing Center (MGHPCC) and connected to Boston University's shared computing cluster network. Image processing pipelines were installed to the system. A high-performance computing system server (large memory, 512GB, two Intel Xeon Gold processors) was added to the existing laboratory dedicated servers (two nodes with GPU computing boxes). The secured data storage with 40TB was also added to the system to maintain the transferred and processed data.

- Data Transfer from BBRAIN: The multi-site dataset was successfully transferred from Boston Biorepository, Recruitment & Integrated Network for GWI (BBRAIN, PI: Sullivan) to our cluster system.

- Processing pipeline: The human connectome project (HCP) style image processing pipeline was installed to the server.

The processing includes,

- Cortical surface modeling and defining regional cortical structures
- Co-registration between structural and diffusion MRI
- Diffusion data preprocessing for correcting motion and eddy current distortions
- Diffusion modeling on following diffusion indices NODDI, generalized q-space imaging (GQI) and DTI (with free water correction) maps.
- Structural connectivity matrix reconstructions and network measures (based on graph-theory).
- Cortical diffusivity mapping
- Diffusion sampling along WM major fiber pathway
- Global Morphometry (i.e., volume, gyrification, etc.)
- Regional Morphometry (i.e., regional volume, thickness, etc.)
- Myelination mapping
- Cortical intensity profile mapping
- Hippocampal subfield volume
- Quality assurance work on the processed results. We performed repeated quality assurance works (i.e., detailed visual inspection, checking modeling errors, distributions, and outliers in the quantification values) on the processed results.

• HCP resting functional connectivity processing. This function performs the following processing steps - bias correction, slice timing correction, motion correction, low-frequency fluctuations extraction, detrending non-neuronal fluctuations in the data, ROI average signal extraction, and connectivity matrix reconstruction

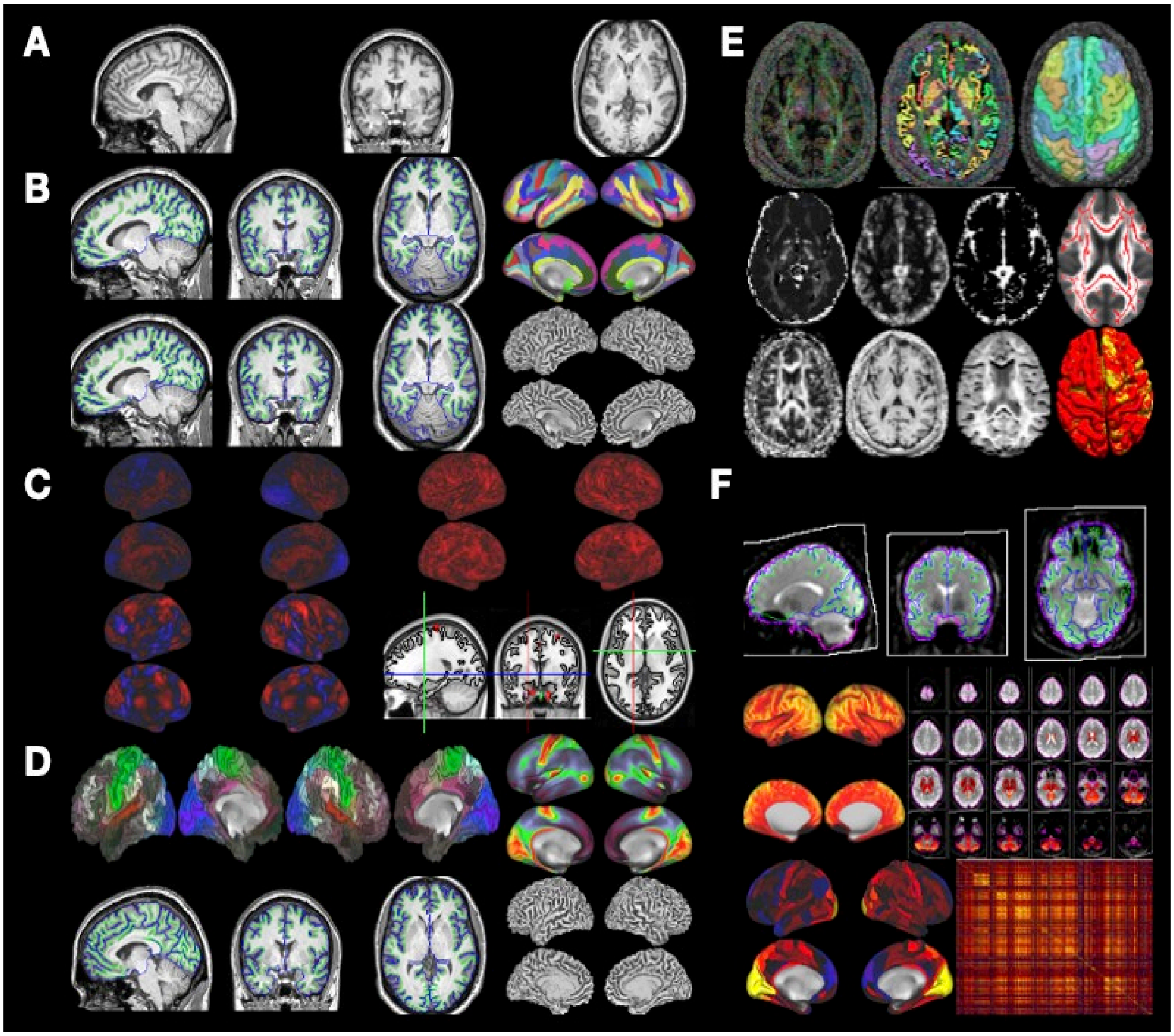

Figure 1. Image processing pipeline. A) T1w anatomical data; B) FreeSurfer Cortical Reconstruction: Tissue segmentation + cortical parcellation + cortical surface mapping; C) Distortion check + Non-linear registration to MNI152 standard space; D) Tissue segmentation in standard space + HCP MMP parcellation (180 ROIs per hemi)+ myelin map; E) Multi-compartment diffusion processing: From top to bottom and from left to right: First row (DSI studio): GQI fiber reconstruction, Freesurfer aparc ROIs overlaid in diffusion space, Freesurfer aparc ROIs surface projection in diffusion space. Second row (NDI and TBSS): ND map, OD map, IsoVF map, TBSS fiber tracking (mean FA skeleton). Third row: Free-water corrected FA map (Free-water model), RDI02 map (DSI studio), mean kurtosis map (dipy dki model), ND map projected into standard surface space; F) Resting state fMRI processing (HCP pipeline): From top to bottom and from left to right: First row: Check surface outlines alignment to MNI standard space and define bounding box. Second row: Processed mean timeseries displayed on inflated surface on the left and subcortical structures displayed in volume view on the right. Third row: HCP MMP-based parcellated timeseries reorganized into resting state network definitions displayed on inflated surfaces on the left, and parcellated timeseries matrix reordered by network definition on the right.

- Image data harmonization techniques: We built a pipeline for running data harmonization techniques for the multisite data. We added three different harmonization functions- 1) linear modeling, 2) Combating batch effect (Combat) harmonization (Beer et al., 2020), 3) Generalized additive model for Combat.

2) Specific objectives:
In the project year 1, we aimed to acquire high quality post-processed data from BBRAIN multisite data and from the external non-veteran population data. Also, we aimed to build a standardized data harmonization protocol.

3) Significant results and Key outcomes:

- Data Transfer from BBRAIN: We summarized the data transfer and processing results (note that some processing works are still on-going).

-

MRI data (processed / Collected)	Boston/Baylor TP1	Boston TP2	Georgetown	Alabama	UCSF 1.5T	UCSF 3T	UCSF 4T
T1 structural	167/167	69/69	147/147	90/90	268/268	196/196	159/159
T2	0/163	0/69	N/A	N/A	N/A	0/194 (Flair, PD, T2)	0/148 (Flair, iPAT2, T2)
pCASL	0/151 (2D)	0/69 (3D)	0/27 (2D)	0/92 (2D ASL)	N/A	N/A	N/A
Diffusion MRI	145/145	69/69	0/143 (35dir b1000 DTI)	N/A	N/A	N/A	N/A
resting-Functional MRI	157/157	67/67	0/149	N/A	N/A	N/A	N/A

Table 1. Multi-site data transferred from BBRAIN. N/A: data not collected. Numbers indicate counts on the transferred and processed data.

- HCP data processing: We completed HCP data morphometric processing. Processing work on HCP diffusion and resting state MRI data is on-going.

- Harmonization pipeline: During the development phase, the pipeline was tested on two different non-veteran datasets from Alzheimer's Disease Neuroimaging Initiative (ADNI) and Framingham Heart Study (FHS). Overall, the Combat based method performed better than the linear methods. We will test those methods to BBRAIN multisite data.

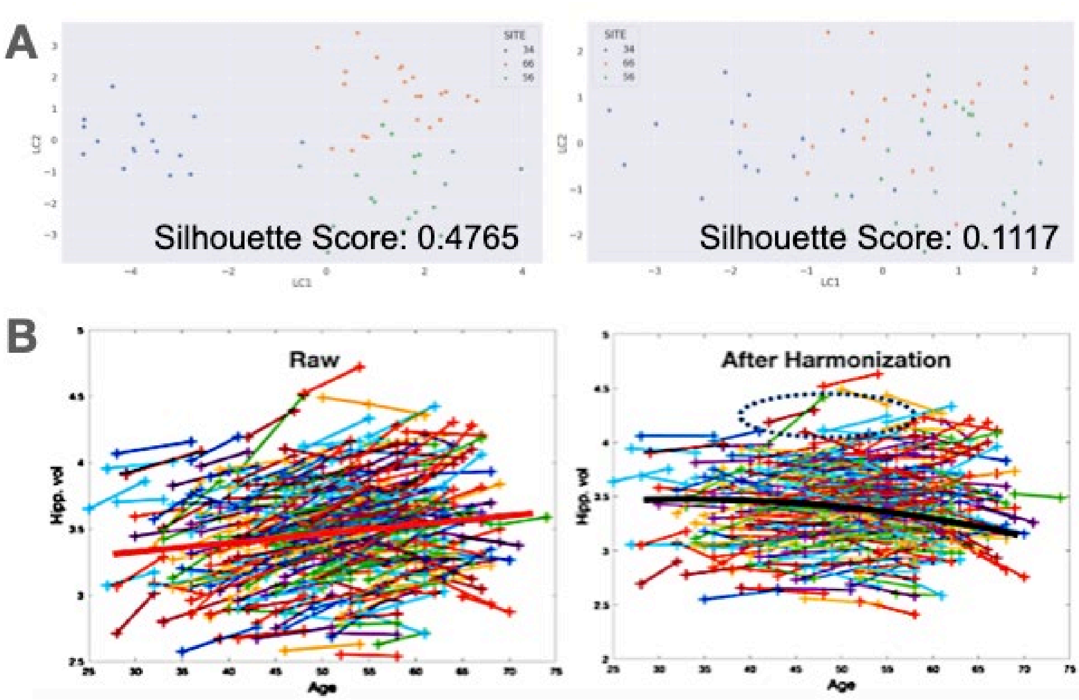

Figure 2. A) Combat harmonization on ADNI data. Silhouette score nearer 0 indicates clusters are overlapped, indicating less site bias. B) Combat harmonization on FHS (longitudinal data). Site-specific biases induced wrong aging trajectory on the hippocampus volumetry and corrected after the harmonization.

4) Other achievements:

- UK biobank data: We plan to take advantage of the extensive multidomain longitudinal large human cohort data of UK biobank to examine GW veteran's brain using UKbiobank's multimodal imaging, clinical and blood biomarker data under the concept of generalized additive models for location, scale and shape (GAMLSS) (Bethlehem et al., 2022). UKbiobank data offers a large-scale data analysis option. Our preliminary analysis using GAMLSS showed that GW cases from two different sites (Boston and San Francisco) have brain profile more distant to the healthy subjects (Fig 3, CN) compared to GW controls. BBRAIN data showed statistically significant

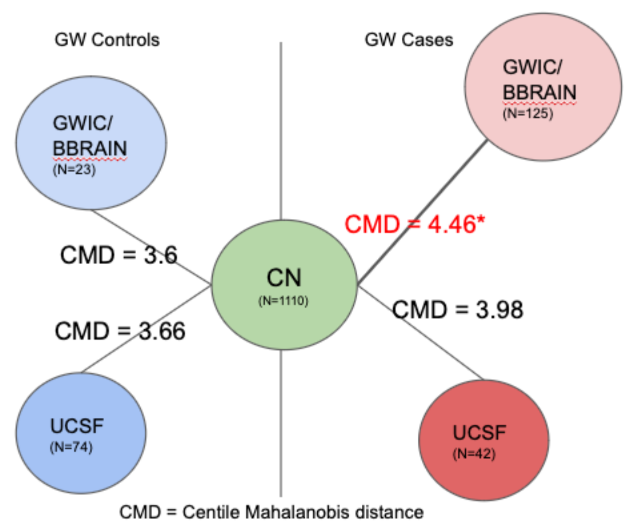

Figure 3. GAMLSS preliminary analysis.

differences compared with BBRAIN or UCSF control veterans in their brain profile measure (Centile Mahalanobis distance).

- Deep learning-based brain age prediction model: We have been testing deep learning based brain age prediction model using multi-model aggregation scheme. Average difference between predicted and actual age (i.e., brain age delta) was less then 1 years when we tested on GWIC data. This is a big improvement compared with our previous attempts. Predicted brain age in veterans will be used together with other imaging derived measure to study GWI specific markers and models in the following project years.

- **What opportunities for training and professional development has the project provided?**

PI has been attending group meetings in BBRAIN (PI: Kimberly Sullivan, co-I in this project) to discuss new ideas and findings.

PI's group has been doing weekly project meetings (every Friday morning).

PI's group has been doing weekly collaboration meetings with Dr.Yangming Ou at Boston Children's hospital to discuss current deep learning topics (every Friday afternoon).

- **How were the results disseminated to communities of interest?**

Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

For the next reporting period, we will continuously work on processing the rest of data. We will also finish testing data harmonization protocol on BBRAIN multisite data. After the harmonization work, we will perform statistical analysis and machine learning modeling.

GW dataset and finalize building the different types of machine learning models to provide efficient computational framework for estimating mTBI and AD risks in GW veterans.

Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

As the project plan on the first year mostly focuses on data preparation and processing, we do not have anything to report yet. However, the processed multisite data, which will be harmonized together in the following years, can be a key resource for the field of GWI research.

- **What was the impact on other disciplines?**

  Nothing to report.

- **What was the impact on technology transfer?**

  Nothing to report.

- **What was the impact on society beyond science and technology?**

  Nothing to report.

__Changes/Problems__

- **Changes in approach and reasons for change**

  Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  Nothing to report.

- **Changes that had a significant impact on expenditures**

  Nothing to report.

__Product__

- **Publications, conference papers, and presentations**

  Nothing to report

- **Journal publications**

  Nothing to report

- **Books or other non-periodical, one-time publications.**

  Nothing to report

- **Other publications, conference papers, and presentations.**

  2023.04.26 Spring 2023 Colloquium, Umass Lowell, invited talk, "*Deciphering Aging Brain: Imaging Markers of Aging & Data Informatics*". Some of the GWI analysis results were shared in this lecture.

Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

Name: *Bang-Bon Koo*
Project Role: *Principal Investigator*

Name: *Kimberley Sullivan*
Project Role: *co-Investigator*

Name: *Dr.Baraniuk*
Project Role: *consultant*

Name: *Jasmine Cheng*
Project Role: *research technician*
Nearest person month worked: *7*
Contribution to Project: *Support on developing the computational resources.*

Name: *Guan Yi*
Project Role: *Graduate student research assistant*
Nearest person month worked: *6*
Contribution to Project: *Support on developing the computational resources.*

Name: *Luis Iberico*
Project Role: *Graduate student research assistant*
Nearest person month worked: *1*
Contribution to Project: *Support on developing the computational resources.*

Name: *Kathakoli Sengupta*
Project Role: *Graduate student research assistant*
Nearest person month worked: *1*
Contribution to Project: *Support on developing the computational resources.*

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Identification of objective brain imaging biomarkers for Gulf War Illness (GWI): exposure- & symptom-specific markers, GW subtypes, and longitudinal patterns
9/01/22-8/31/25
Cal mos.: 2.2
Role: PI

Confirmation of the Low Glutamate Diet as a Treatment for Gulf War Illness
09/01/2022 - 08/31/2025
Cal mos.: 0.6
Role: co-I

Are Tau Proteins in Blood and PET Images Related to Gulf War Illness and Risk of Comorbid Neurological Disorders?
9/01/22-8/31/25
Cal mos.: 1.2
Role: co-I

Installation of a novel *in vivo* BBB imaging protocol to study complex brain aging patterns of AD.
1/1/23-12/31/23
Cal mos.: 1.6
Role: PI

- **What other organizations were involved as partners?**

Nothing to report

Special Reporting Requirements

- **COLLABORATIVE AWARDS:**

Nothing to report

- **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

Nothing to report

Appendices

Bethlehem, R.A.I., Seidlitz, J., White, S.R. *et al.* Brain charts for the human lifespan. *Nature* **604**, 525–533 (2022). https://doi.org/10.1038/s41586-022-04554-y

Beer, J. C., Tustison, N. J., Cook, P. A., Davatzikos, C., Sheline, Y. I., Shinohara, R. T., ... & Alzheimer's Disease Neuroimaging Initiative. (2020). Longitudinal ComBat: A method for harmonizing longitudinal multi-scanner imaging data. *Neuroimage*, *220*, 117129.