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Additional value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma: methodology and potential clinical implications of an Italian multicentric trial.

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ABSTRACT

Introduction: Assessment of response to therapy in pediatric Hodgkin lymphoma (HL) patients by 18F-fluorodeoxyglucose PET/CT (FDG PET) has become a powerful tool for the discrimination of responders from non-responders. The addition of volumetric and texture analyses can be regarded as a valuable help for disease prognostication and biological characterization. Based on these premises, the AIEOP Hodgkin Lymphoma Study Group has designed a prospective evaluation of volumetric and texture analysis in the Italian cohort of patients enrolled in the EuroNet-PHL-C2.

Methods and Analysis: The primary objective is to compare volumetric assessment in HL patients at baseline and during the course of therapy with standard visual and semi-quantitative analyses. The secondary objective is to identify the impact of volumetric and texture analysis on bulky masses. The tertiary objective is to determine the additional value of multiparametric assessment in patients having a partial response on morphological imaging.

The overall cohort of the study is expected to be round 400-500 patients, with approximately half presenting with bulky masses. All PET scans of the Italian cohort will be analyzed for volumetric assessment, comprising metabolic tumor volume (MTV) and total lesion glycolysis (TLG) at baseline and during the course of therapy. A dedicated software will delineate semi-automatically contours using different threshold methods, and the impact of each segmentation techniques will be evaluated. Bulky will be defined on contiguous lymph node masses ≥200ml on CT/MRI. All bulky masses will be outlined and analyzed by the same software to provide textural features. Morphological assessment will be based in RECIL 2017 for response definition.

Ethics and Dissemination: The current study has been ethically approved (AIFA/SC/P/27087 approved 09/03/2018; EudraCT 2012-004053-88, EM-04). The results of the different analyses performed during and after study completion the will be actively disseminated through peer-reviewed journals, conference presentations, social media, print media and internet.

Keywords: FDG PET; Hodgkin's lymphoma; pediatric; volumetric analysis; response assessment; texture analysis; bulky masses; interim evaluation.

BACKGROUND

18F-fluorodeoxyglucose positron emission tomography (FDG PET) has become a standard diagnostic procedure for the assessment of response to therapy in adults and children with Hodgkin lymphoma. International guidelines recommend the using of Deauville five-point scale as a visual method for discriminating responders from non-responder patients [**1, 2**]. In 2014, the pediatric German group proposed the use of qPET with the intent to extend the Deauville score to a continuous scale and limit optical misinterpretation due to the influence of background activity [**3, 4**]. This quantitative method is being applied in the current EuroNet-PHL-C2 clinical trial, in which adapted therapy is based on quantitative FDG avidity of tumor masses on PET evaluation after 2 cycles of OEPA [**5, 6**]. This approach, however, postpones risk stratification at interim evaluation; therefore, the definition of imaging baseline predictors is highly desirable.

The implementation metabolic tumor volume, as a sum of areas with an increased SUV inside the tumor, as well as the characterization of the heterogeneity of tumor metabolic patterns on FDG PET has become an emerging topic in nuclear medicine **[7**]. Several studies [**8-11**] have shown that the addition of volumetric and textural parameters can be a valuable help for disease prognostication and biological characterization of many tumor types, thus suggesting a similar implication for pediatric Hodgkin lymphoma [**12**]. On the other hand, the scientific background and the results obtained from our previous studies in the context of the Italian AIEOP-LH2004 trial [**13, 14**] suggest an additional impact of FDG PET in patients with or without bulky disease presenting with residual masses on morphological evaluation with computed tomography (CT).

Given the abovementioned premises, the AIEOP Hodgkin Lymphoma Study Group has planned to perform in the Italian cohort of patients treated according to the EuroNet-PHL-C2 trial additional volumetric analyses to improve the evaluation of tumor burden computed at baseline FDG PET and to identify prognostic factors suitable for predicting early metabolic response to therapy in pediatric Hodgkin lymphoma (HL). In case of bulky disease, further textural and shape analysis in the baseline FDG PET will be performed to evaluate macroscopic and microscopic heterogeneity of tumor masses, as reflection of their aggressiveness and different sensibility to chemotherapy.

STUDY OBJECTIVES

This is a prospective observational multicentric cohort study. The primary objective of the study is to compare the diagnostic and prognostic role of volumetric assessment in HL patients at baseline and during the course of therapy with standard visual (Deauville score) and semi-quantitative (i.e. SUVmax, SUVmean, SUVpeak) analyses.

The secondary objective of the study is to identify the diagnostic and prognostic impact of texture analyses and the other metabolic parameters on bulky masses.

The tertiary objective of the study is to determine the additional predictive and prognostic value of multiparametric assessment (i.e. SUVmax, SUVmean, SUVpeak, MTV, TLG and texture analysis) in HL patients having a partial response on morphological imaging.

ELIGIBILITY CRITERIA

In accordance to the EuroNet-PHL-C2 trial, the population of our study will include pediatric patients <18 years of age, with histologically confirmed primary diagnosis of classical Hodgkin's lymphoma, who will undergo FDG PET at baseline (PET1), after two cycles of two cycles of induction OEPA therapy (PET2), and after the end of chemotherapy (PET3), in case of PET2 positive patients [**15**]. Patients will be stratified at baseline in one of the three Treatment Levels (TL) on basis of stage and risk factors, confirmed by central review: TL-1, TL-2, TL-3 for low, intermediate and advanced HL, respectively [**6, 15**].

METHODOLOGY

Whole body assessment of HL

All FDG PET scans performed in the Italian cohort of patients undergoing the EuroNet-PHL-C2 trail will be analyzed with additional volumetric assessment comprising metabolic tumor volume (MTV) and total lesion glycolysis (TLG) at baseline and during the course of therapy.

In each patient, HL lesions will be identified by visual analysis and corresponding SUVmax, SUVmean and SUVpeak [**16**] will be determined as the pixel with the highest value of uptake, the mean value of uptake and the average value of uptake in a VOI (volume of interest) of 1ml that surrounds the voxel with the highest activity, respectively. A dedicated software will be used to delineate, semi-automatically, contours of the lesions using different threshold methods and the impact of segmentation technique will be evaluated (**Figure 1**). More specifically, four threshold methods will be used based on previously reported methodologies [**17-20**]:

- Fixed 41% threshold of the SUVmax within the respective lymphoma site,
- Fixed absolute SUV threshold of 2.5;
- SUVmax(lesion)/SUVmean liver >1.5
- Adaptative method: I(threshold)= [0.15 x I(mean)]+ I(background). I(mean) is calculated as the mean intensity of all pixels surrounded by the 70% Imax isocontour within the tumor; I(background) is defined as a SUVmean of liver [**21**].

After delineation of all individual lesions, patient MTV will be estimated as the sum of voxels with supra-threshold uptake, reported in ml, and TLG will be calculated as [MTV x SUVmean].

PET2 scans will be evaluated by visual analysis on the basis of Deauville-5-points-scale assigning Inadequate Response (IR) when at least one site shows FDG uptake higher than liver uptake (scores 4 and 5). Additionally, the variation of SUVmax, determined as the percentage reduction between the SUVmax in the tumor site with the most intense uptake on PET1 and the SUVmax in the tumor site with the most intense uptake on PET2 (ΔSUVmax) [**9**], will be computed. Similarly, will be calculated the variation of SUVmean, SUVpeak, MTV, and TLG, respectively.

Assessment of bulky masses

The definition of bulky masses will be determined as specified in EuroNet-PHL-C2 [**15**]. More specifically, a volume of a contiguous lymph node mass ≥ 200ml, measured by the three largest diameters on CT/MRI, will be considered as bulky. All bulky masses will be outlined using different threshold methods, as explained above, and analyzed on dedicated software for semi-quantitative and volumetric parameters. The same software will provide textural and shape features.

SUVmax will be defined as the maximum uptake in the segmented tumor. SUVmean will be measured as the average uptake in the tumor burden. SUVpeak will be computed as the average SUV in a 1ml region of tumor burden around the maximal SUV voxel. MTV will be the volume of the segmented tumor. TLG will be calculated as the product of SUVmean by MTV.

Among shape parameters, asphericity, convexity and 3D fractal dimensions will be computed [**15, 18, 22, 23**]. For the characterization of tumor texture, two methods will be used as previously reported [**11, 24, 25**]: analysis of the histogram of the voxel values within the tumor and the method accounting for the spatial arrangement of voxel values. By histogram-based method, on first-order statistics, will be computed SD (standard deviation), Entropy, Energy, kurtosis and Skewness. To define the spatial arrangement of the voxel values within the tumor, four matrices will be computed from each VOI: gray-level co-occurance matrix (GLCM), neighborhood gray-level different matrix (NGLDM), gray-level zone length matrix (GLZLM) and gray-level run length matrix (GLRLM).

Definition of morphological response

In pediatric HL patients presenting with morphological partial response on bulky masses and/or residual lymph nodes with largest diameter ≥ 2 cm, a multiparametric assessment (i.e. SUVmax, SUVmean, SUVpeak, MTV, TLG and texture analysis) will be performed. For this purpose, the International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017) will be used when necessary [**26**]. In particular, we will include in the analysis all cases with:

- Poor bulk response: < 50% volume reduction and/or at least one nodal site with largest diameter of ≥ 2 cm and non-assessable qPET-value due to brown fatty tissue [**15**].
- Partial Response: ≥30% decrease in the sum of longest diameters of target lesions but no complete response; positive PET (DS 4–5); any bone marrow involvement, no new lesions [**26**].
- Minor Response: ≥ 10% decrease in the sum of longest diameters of target lesions but not a partial response [**26**].

Sample size calculation

Given the limited number of robust data for volumetric and texture analysis in pediatric HL population, we considered adequate a sample size comprising all eligible patients. In the current study, we aspect to enroll minimum 50-80 patients per year from the Italian Hodgkin Lymphoma Group out for the different AIEOP Italian Centers. Based on the data derived from the previous AIEOP-LH2004 trial, the estimated number of bulky masses is quoted around 50% of the enrolled cases. Consequently, the overall cohort to be included in the study is expected of round 400-500 patients, with half presenting with bulky masses, eligible for dedicated analyses.

PATIENT AND PUBLIC INVOLVEMENT

No patient involved

STATISTICAL ANALYSIS

Descriptive statistics will be performed using conventional metrics (mean, median, range). All metabolic and heterogeneity parameters will be correlated with each other and with the disease outcome and their diagnostic and prognostic role will be investigated. For continuous data, differences between groups will be compared by the T test or the Wilcoxon test, when appropriate. For rank correlation, we will use Spearman' correlation coefficient (rho). The different threshold methods used to outline all individual lesions will be compared by the Pearson correlation coefficient, linear regression, Bland-Altmann and logistic regression. Optimal cut-off values of the metabolic parameters and, in patients with bulky mass, also of textural parameters for distinguishing inadequate response (IR) from adequate response (AR) to therapy will be defined by receiver operating characteristic (ROC) curves with respective areas under the curve (AUC). Patients with or without bulky mass will be divided into groups of complete metabolic response (CMR), partial metabolic response (PMR), no metabolic response (NMR) and progressive metabolic disease (PMD) and differences in metabolic and textural parameters will be investigated

by analysis of variance (ANOVA). Linear regression will be applied to determine the relationship between response and all other variables. Statistical significance will be set for $p < 0.05$.

DISCUSSION

In literature there is evidence that metabolically active tumor volume determined by PET/CT is more advantageous than tumor volume measured by CT or MRI for predicting response to treatment in various malignancies afflicting both adult and the pediatric population. More specifically, in adult population, recent publications have demonstrated that the measurement of 3-dimensional disease volume (MTV) and metabolic activity (TLG) [**27**] can help predict outcomes in HL patients [**8, 9, 27-29**]. This might suggest a similar implication also in pediatric HL, where the tumor volume may not change because of overlapping inflammatory processes correlated to therapy, while early changes of metabolic activity are most frequently reported.

Along with the above mentioned semi-quantitative parameters, it is possible to extract other quantitative features from PET-CT images, including intensity, heterogeneity, and shape within the tumor, potentially reflecting underlying biological characteristics [**27**]. These characteristics are embedded in the so called "Radiomics", a translational field of research aiming to extract high-dimensional data from clinical images to predict underlying biological characteristics of the disease [**30**] . Radiomic features are correlated to prognostic markers in cancer (i.e. hypoxia, angiogenesis, proliferation, etc.) and might be utilized for tumor response prediction and outcome prognostication. In pediatric population, especially in case of advanced stage disease, high dose therapeutic regimens represent the standard to guarantee cure, yet at the expense of early and delayed side effects [**31**]. In this context, it becomes even more important to identify those factors capable of limiting the doses to the necessary therapeutic effect while reducing at maximum the undesirable consequences. These prerogatives have guided in the last decades clinical research in adult [**32, 33**] and pediatric HL [**6, 15**].

In the present study, we aim to identify prospectively the role of volumetric and texture (radiomic) characteristics better fulfilling the need for predictive and prognostic factors in pediatric HL. Thanks to a large sample size and to a preliminary methodological validation, we expect to obtain significant data on the added value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma.

STRENGTHS AND LIMITATIONS OF THE STUDY

- This study will represent the largest analysis on volumetric and semi-quantitative parameters in pediatric HL undergoing a therapeutic trial.
- The dedicated evaluation of texture features in HL bulky masses, will allow for a solid definition of the impact of radiomics in this large pediatric population.
- Thanks to a comparative disease evaluation with both metabolic (PET) and morphological (CT/MRI) parameters, we will be able to assess the added value of the technique in HL patients presenting with a partial response to therapy.
- Since all study examinations are centrally reviewed after online platform upload of DICOM images, the only limitation of the study is related to the retrieval of all uploaded scans.

LIST OF ABBREVIATIONS

AIEOP = Associazione Italiana di Ematologia e Oncologia Pediatrica

CT = computed tomography

DS = Deauville score

FDG = 18F-fluorodeoxyglucose

GLCM = gray-level co-occurance matrix

GLRLM = gray-level run length matrix

GLZLM = gray-level zone length matrix

HL = Hodgkin lymphoma

MRI = magnetic resonance imaging

MTV = metabolic tumor volume

NGLDM = neighborhood gray-level different matrix

OEPA = Vincristine Sulfate (Oncovin), Etoposide Phosphate, Prednisone. Doxorubicin Hydrochloride (Adriamycin)

PET = positron emission tomography

SD = standard deviation

SUV = standardized uptake value

TLG = total lesion glycolysis

VOI = volume of interest

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The current study has been approved by AIFA (Agenzia Italiana del Farmaco) the 9th of March 2018 (EudraCT 2012-004053-88, EM-04; AIFA/SC/P/27087 approved 09/03/2018). All procedures involving human participants will be performed in accordance with the ethical standards of the institutional and/or national research committee and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All patients will sign a written informed consent to participate in the study.

CONSENT FOR PUBBLICATION

Not applicable

AVAILABILIY OF DATA AND MATERIAL

Not applicable

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

AUTHORS' CONTRIBUTION

EL, CE, RB and MM planned, coordinated and conducted the study. Medical care is covered by the AIEOP Centers for the Hodgkin Lymphoma Study Group. Scientific program is planned by MM, EL, RB. All authors read and approved the final manuscript.

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REFERENCES

1. Cheson, B.D., et al. Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma: The Lugano Classification. *J Clin Oncol.* 2014, Vol. 32, 27, p. 3059-67.

2. Barrington, S.F., et al. Role of Imaging in the Staging and Response Assessment of Lymphoma: Consensus of International Conference on Malignant Lymphomas Imaging Working Group. *J Clin Oncol.* 2014, Vol. 32, 27, p. 3048-58.

3. Barrington, S.F., et al. FDG PET for therapy monitoring in Hodgkin and non-Hodgkin lymphoma. *Eur J Nucl Med Mol Imaging.* 2017, Vol. 44, p. 97-110.

4. Hasenclever D., et al. qPET – a quantitative extension of the Deauville scale to assess response in iterim FDG-PET scans in lymphoma. *Eur J Nucl Med Mol Imaging.* 2014, Vol. 41, p. 1301-8.

5. Mauz-Körholz C., et al. Procarbazine-free OEPA-COPDAC chemotherapy in boys and standard OPPA-COPP in girls have comparable effectiveness in pediatric Hodgkin's lymphoma: the GPOH-HD-2002 study. *J Clin Oncol.* 2016, Vol. 28, 23, p. 3680-6.

6. Rogasch JMM, et al. Pretherapeutic FDG-PET total metabolic tumor volume predicts response to induction therapy in pediatric Hodgkin's lymphoma. *BMC Cancer*. 2018; Vol. 18: p.521

7. Kostakoglu, L., et al. FDG-PET imaging for Hodgkin Lymphoma: Currente use and future applications. *Clin Adv Hematol Oncol.* 2014, Vol. 12, p. 20-35.

8. Meignan, M., et al. Metabolic tumou volumes measured at staging in lymphoma: Methodological evaluation on phantom experiments and patients. *Eur J Nucl Med Mol Imaging.* 2014, Vol. 41, p. 1113-1122.

9. Kanoun, S., et al. Baseline metabolic tumour volume is an indipendent prognostic factor in Hodgkin lymphoma. *Nucl Med Mol Imaging.* 2014, Vol. 41, p. 1735-1743.

10. Brooks, F.J., et al. Low-order non-spatial effects dominate second-order spatial effects in the texture quantifier analysis of 18F-FDG-PET images. *PLoS One.* 2015, Vol. 10, p. e0116574.

11. Buvat, I., et al. Tumor texture analysis in PET: Where do we stand? *J Nucl Med.* 2015, Vol. 56, p. 1642-44.

12. Kluge, R., et al. Current Role of FDG-PET in Pediatric Hodgkin's Lymphoma. *Semin Nucl Med.* 2017, Vol. 47, p. 242-257.

13. Lopci, E., et al. Postchemotherapy PET evaluation correlates with patient outcome in paediatric Hodgkin's disease. *Eur J Nucl Med Mol Imaging.* 2011, Vol. 38, 9, p. 1620-7.

14. Lopci, E., et al. Additional role of FDG-PET in paediatric HD patients presenting with a partial response on CT: data deriving from a multicentric Italian study. *Eur J Nucl Med Mol Imaging.* 2012, Vol. 39, p. S155–S303.

15 . Second International Inter-Group Study for Classical Hodgkin Lymphoma in Children and Adolescents. Final protocol version, 1st amendment. https://clinicaltrials.gov/ct2/show/NCT02684708.

16. Vanderhoek, M., et al. Impact of the Definition of Peak Standardized Uptake Value on Quantification of Treatment Response. *J Nucl Med.* 2012, Vol. 53, p. 4-11.

17. Boellaard, R., et al. FDG PET and PET/CT: EANM procedure guidelines for tumourPET imaging: version 1.0. *Eur J Nucl Med Mol Imaging.* 2010, Vol. 37, p. 181-200.

18. Ben Bouallègue, F., et al. Association between textural and morphological tumor indices on baseline PET-CT and early metabolic response on interim PET-CT in bulky malignant lymphomas. *Med Phys.* 2017, Vol. 44, p. 4608-19.

19. Mettler, J., et al. Metabolic Tumour Volume for Response Prediction in Advanced-Stage Hodgkin Lymphoma. *J Nucl Med.* 2018.

20. Cottereau, A.S., et al. Prognostic value of baseline metabolic tumor volume in early-stage Hodgkin lymphoma in the standard arm of the H10 trial. *Blood.* 2018, Vol. 131, p. 1456-63.

21. Nestle U., et al. Comparison of different methods for delineation of 18F-FDG PET-positive tissue for target volume definition in radiotherapy of patients with non-Small cell lung cancer. *J Nucl Med*. 2005; Vol. 46: p.1342-8.

22. Castello, A., et al. Prognostic Impact of Intratumoral Heterogeneity Based on Fractal Geometry Analysis in Operated NSCLC Patients. *Mol Imaging Biol.* 2018.

23. Grizzi, F., et al. The Complexity and Fractal Geometry of Nuclear Medicine Images. *Mol Imaging Biol.* 2018.

24. Orlhac, F., et al. Tumor Texture Analysis in 18F-FDG PET: Relationships Between Texture Parameters, Histogram Indices, Standardized Uptake Values, Metabolic Volumes, and Total Lesion Glycolysis. *J Nucl Med.* 2014, Vol. 55, p. 414-422.

25. Ganeshan, B., et al. CT-based texture analysis potentially provides prognostic information complementary to interim fdg-pet for patients with hodgkin's and aggressive non-hodgkin's lymphomas. *Eur Radiol.* 2017, Vol. 27, p. 1012-1020.

26. Younes A. et al, International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017). *Ann Oncol*. 2017;Vol. 28: P. 1436-1447.

27. Milgrom et al., A PET radiomics model to predict refractory mediastinal Hodgkin Lymphoma. *Sci Rep* 2019; Vol. 9; p. 1322.

28. Song MK. et al., Metabolic tumor volume by positron emission tomography/computed tomography as a clinical parameter to determine therapeutic modality for early stage Hodgkin's lymphoma. Cancer Sci 2013; Vol. 104: p. 1656-61.

29. Akhtari M., et al. Reclassifying patients with early-stage Hodgkin lymphoma based on functional radiographic markers at presentation. Blood. 2018; Vol. 131: p. 84-94.

30. Rizzo S, et al. Radiomics: the facts and the challenges of image analysis. Eur Radiol Exp 2018;2:36.

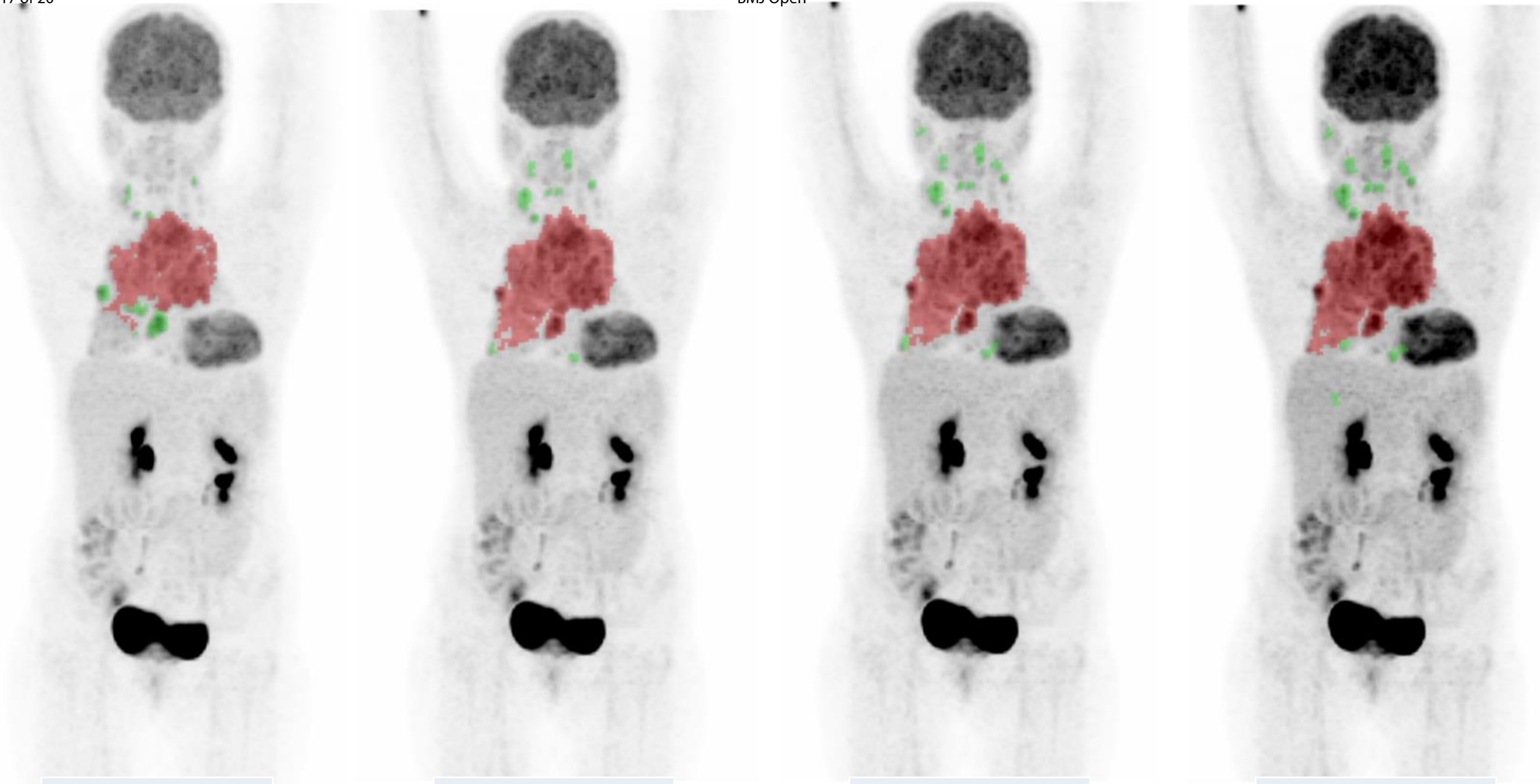
31. Lackner H., et al, Prospectiveevaluation of late effects after childhood cancer therapy with follow-up over 9 years. *Eur J Pediatr* 2000; Vol. 159: p. 750-758.

32. Borchmann P, et al. PET-guided treatment in patients with advanced-stage Hodgkin's lymphoma (HD18): final results of an open-label, international, randomised phase 3 trial by the German Hodgkin study group. Lancet. 2018;Vol. 390: p. 2790–802.

33. Casasnovas RO, et al. PET-adapted treatment for newly diagnosed advanced Hodgkin lymphoma (AHL2011): a randomised, multicentre, non-inferiority, phase 3 study. *Lancet Oncol* 2019; Vol. 20: p. 202-215.

FIGURE LEGEND

Figure 1: Comparative representation of the four segmentation techniques applied in our study protocol illustrated from left to right: fixed 41% threshold (V41%); fixed absolute SUV threshold of 2.5 (V2.5); SUVmax(lesion)/SUVmean liver >1.5 (Vliver); and adaptative method (AM). The same HL patient has been analyzed according to the above mentioned techniques and corresponding TMTV (total metabolic tumor volumes) at baseline have been displayed for comparison.



TMTV (V41%) Baseline	TMTV (V2.5) Baseline	TMTV (Vliver) Baseline	TMTV (AM) Baseline
242,30	471,00	500,40	538,3

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Additional value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma in the context of the euronet-PHL-C2-Trial.
Trial registration	2a	EudraCT 2012-004053-88, EM-04;
	2b	AIFA/SC/P/27087 approved 9th of March 2018
Protocol version	3	Amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Funding	4	Declarations, article page 10
Roles and responsibilities	5a	Responsible investigators: Egest Lopci, MD, PhD, Nuclear Medicine department, Humanitas Clinical and Research Hospital – IRCCS, Rozzano (MI), Italy Maurizio Mascarin, MD, AYA and Pediatric Radiotherapy IRCCS Centro di Riferimento Oncologico Roberta Burnelli, MD, Pediatric Onco-hematologic Unit, University Hospital S. Anna, Ferrara Caterina Elia, AYA and Pediatric Radiotherapy, IRCCS Centro di Riferimento Oncologico Arnoldo Piccardo, MD, Nuclear Medicine department, Galliera Hospital, Genoa, Italy. Eugenio Borsatti, MD, Nuclear Medicine department, Centro di Riferimento Oncologico, Aviano, Pordenone, Italy Pietro Zucchetta, MD, Nuclear Medicine Department, University Hospital, Padova, Italy Angelina Cistaro, MD, Positron Emission Tomography Centre, IRMET S.p.A. Affidea, Turin, Italy
	5b	Maurizio Mascarin, MD, AYA and Pediatric Radiotherapy IRCCS Centro di Riferimento Oncologico
	5c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
	5d	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Introduction		

Background and rationale	6a	Background, article page 4
	6b	Background, article page 4
Objectives	7	Study objectives, article page 4
Trial design	8	This is a prospective observational multicentric cohort study.

Methods: Participants, interventions, and outcomes

Study setting	9	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Eligibility criteria	10	Eligibility criteria, article page 5
Interventions	11a	N/A
	11b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
	11c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
	11d	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Outcomes	12	Study objectives, article page
Participant timeline	13	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Sample size	14	Sample size calculation, article page 7
Recruitment	15	AIEOP Hodgkin Lymphoma Study Group

Methods: Assignment of interventions (for controlled trials)

Allocation:		
Sequence generation	16a	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Allocation concealment mechanism	16b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Implementation	16c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Blinding (masking)	17a	Open label

	17b	N/A
Methods: Data collection, management, and analysis		
Data collection methods	18a	Statistical Analysis, article page 7
	18b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Data management	19	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Statistical methods	20a	Statistical Analysis, article page 7
	20b	Statistical Analysis, article page 7
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Methods: Monitoring		
Data monitoring	21a	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
	21b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Harms	22	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Auditing	23	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Ethics and dissemination		
Research ethics approval	24	The protocol has been already board (REC/IRB) approved
Protocol amendments	25	N/A
Consent or assent	26a	Local investigators affiliated to the AIEOP (Associazione Italiana Ematologia Oncologia Pediatrica) centers.
	26b	N/A
Confidentiality	27	As per EuroNet-PHL-C2 protocol and AIEOP policy
Declaration of interests	28	Declarations, article page 10
Access to data	29	As per EuroNet-PHL-C2 protocol and AIEOP policy

Ancillary and post-trial care	30	N/A
Dissemination policy	31a	Trial results will be communicated to participants, healthcare professionals, the public, and other relevant groups via scientific and congress publications.
	31b	Authorship eligibility will be provided to investigators based on their contribution to the study.
	31c	As per EuroNet-PHL-C2 protocol and AIEOP policy
Appendices		
Informed consent materials	32	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Biological specimens	33	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Additional value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma: methodology and potential clinical implications of an Italian multicentric trial.

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Additional value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma: methodology and potential clinical implications of an Italian multicentric trial.

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ABSTRACT

Introduction: Assessment of response to therapy in pediatric Hodgkin lymphoma (HL) patients by 18F-fluorodeoxyglucose PET/CT (FDG PET) has become a powerful tool for the discrimination of responders from non-responders. The addition of volumetric and texture analyses can be regarded as a valuable help for disease prognostication and biological characterization. Based on these premises, the AIEOP Hodgkin Lymphoma Study Group has designed a prospective evaluation of volumetric and texture analysis in the Italian cohort of patients enrolled in the EuroNet-PHL-C2.

Methods and Analysis: The primary objective is to compare volumetric assessment in HL patients at baseline and during the course of therapy with standard visual and semi-quantitative analyses. The secondary objective is to identify the impact of volumetric and texture analysis on bulky masses. The tertiary objective is to determine the additional value of multiparametric assessment in patients having a partial response on morphological imaging.

The overall cohort of the study is expected to be round 400-500 patients, with approximately half presenting with bulky masses. All PET scans of the Italian cohort will be analyzed for volumetric assessment, comprising metabolic tumor volume (MTV) and total lesion glycolysis (TLG) at baseline and during the course of therapy. A dedicated software will delineate semi-automatically contours using different threshold methods, and the impact of each segmentation techniques will be evaluated. Bulky will be defined on contiguous lymph node masses ≥200ml on CT/MRI. All bulky masses will be outlined and analyzed by the same software to provide textural features. Morphological assessment will be based in RECIL 2017 for response definition.

Ethics and Dissemination: The current study has been ethically approved (AIFA/SC/P/27087 approved 09/03/2018; EudraCT 2012-004053-88, EM-04). The results of the different analyses performed during and after study completion the will be actively disseminated through peer-reviewed journals, conference presentations, social media, print media and internet.

Keywords: FDG PET; Hodgkin's lymphoma; pediatric; volumetric analysis; response assessment; texture analysis; bulky masses; interim evaluation.

STRENGTHS AND LIMITATIONS OF THE STUDY

- This study will represent the largest analysis on volumetric and semi-quantitative parameters in pediatric HL undergoing a therapeutic trial.
- The dedicated evaluation of texture features in HL bulky masses, will allow for a solid definition of the impact of radiomics in this large pediatric population.
- Thanks to a comparative disease evaluation with both metabolic (PET) and morphological (CT/MRI) parameters, we will be able to assess the added value of the technique in pediatric HL patients presenting with a partial response to therapy.
- Since all study examinations are centrally reviewed after online platform upload of DICOM images, one limitation of the study is related to the effective retrieval of all uploaded scans.
- The segmentation used for volumetric analyses can be considered a limit, since the predefined threshold methods might not be applicable for all lesions, particularly during response assessment; hence, a preliminary validation study will be performed with this regards.
- Data extraction for radiomic features will be necessarily performed in PET exams obtained from different scanners and undergoing different reconstruction algorithms, although harmonization based on EANM guidelines is recommended for the trial.

BACKGROUND

18F-fluorodeoxyglucose positron emission tomography (FDG PET) has become a standard diagnostic procedure for the assessment of response to therapy in adults and children with Hodgkin lymphoma. International guidelines recommend the using of Deauville five-point scale as a visual method for discriminating responders from non-responder patients [**1, 2**]. In 2014, the pediatric German group proposed the use of qPET with the intent to extend the Deauville score to a continuous scale and limit optical misinterpretation due to the influence of background activity [**3, 4**]. This quantitative method is being applied in the current EuroNet-PHL-C2 clinical trial, in which adapted therapy is based on quantitative FDG avidity of tumor masses on PET evaluation after 2 cycles of OEPA [**5, 6**]. This approach, however, postpones risk stratification at interim evaluation; therefore, the definition of imaging baseline predictors is highly desirable.

The implementation of metabolic tumor volume, as a sum of areas with an increased SUV inside the tumor, as well as the characterization of the heterogeneity of tumor metabolic patterns on FDG PET has become an emerging topic in nuclear medicine **[7**]. Several studies [**8-11**] have shown that the addition of volumetric and textural parameters can be a valuable help for disease prognostication and biological characterization of many tumor types, thus suggesting a similar implication for pediatric Hodgkin lymphoma [**12**]. While the concept of "Radiomics", consisting on the extraction of a large quantity of features from digital images via data-characterization algorithms has gained a proper place in predicting outcome and early metabolic response in adults with malignant lymphoma [**13, 14**] On the other hand, the scientific background and the results obtained from our previous studies in the context of the Italian AIEOP-LH2004 trial [**15, 16**] suggest an additional impact of FDG PET in patients with or without bulky disease presenting with residual masses on morphological evaluation with computed tomography (CT).

Given the abovementioned premises, the AIEOP Hodgkin Lymphoma Study Group has planned to perform in the Italian cohort of patients treated according to the EuroNet-PHL-C2 trial additional volumetric analyses to improve the evaluation of tumor burden computed at baseline FDG PET and to identify prognostic factors suitable for predicting early metabolic response to therapy in pediatric Hodgkin lymphoma (HL). In case of bulky disease, further textural and shape analysis in the baseline FDG PET will be performed to evaluate macroscopic and microscopic heterogeneity of tumor masses, as reflection of their aggressiveness and different chemotherapy sensitivity.

STUDY OBJECTIVES

This is a prospective observational multicentric cohort study. The primary objective of the study is to compare the diagnostic and prognostic role of volumetric assessment in HL patients at baseline

and during the course of therapy with standard visual (Deauville score) and semi-quantitative (i.e. SUVmax, SUVmean, SUVpeak) analyses.

The secondary objective of the study is to identify the diagnostic and prognostic impact of texture analyses and the other metabolic parameters on bulky masses.

The tertiary objective of the study is to determine the additional predictive and prognostic value of multiparametric assessment (i.e. SUVmax, SUVmean, SUVpeak, MTV, TLG and texture analysis) in HL patients having a partial response on morphological imaging.

ELIGIBILITY CRITERIA

In accordance to the EuroNet-PHL-C2 trial, the population of our study will include pediatric patients of the Italian cohort, aged <25 years, with histologically confirmed primary diagnosis of classical Hodgkin's lymphoma, who will undergo FDG PET at baseline (PET1), after two cycles of induction OEPA therapy (PET2), and after the end of chemotherapy (PET3), in case of PET2 positive patients [**17**]. Patients will be stratified at baseline in one of the three Treatment Levels (TL) on basis of stage and risk factors, confirmed by central review: TL-1, TL-2, TL-3 for low, intermediate and advanced HL, respectively [**6, 17**].

STUDY TIMELINE

The protocol herein illustrated represents a parallel study on PET imaging performed after the EuroNet-PHL-C2 trial amendment (Amendment Nr. 04, dated 2017-07-31) on the Italian cohort of patients. The study has been also submitted and approved by the Italian authority (AIFA) the date 2018-03-09. Consequently, the timeline of the protocol will be as follows: I) enrollment period will start from the 10th of March 2018 until 31st of December 2020; II) follow-up period will last 5 years after last enrolment day; III) study completion is planned before 31st December 2025.

METHODOLOGY

Whole body assessment of HL

All FDG PET scans performed in the Italian cohort of patients undergoing the EuroNet-PHL-C2 trail will be analyzed with additional volumetric assessment comprising metabolic tumor volume (MTV) and total lesion glycolysis (TLG) at baseline and during the course of therapy.

In each patient, HL lesions will be identified by visual analysis and corresponding SUVmax, SUVmean and SUVpeak [**18**] will be determined as the pixel with the highest value of uptake, the mean value of uptake and the average value of uptake in a VOI (volume of interest) of 1ml that surrounds the voxel with the highest activity, respectively. A dedicated software will be used to

delineate, semi-automatically, contours of the lesions using different threshold methods and the impact of segmentation technique will be evaluated (**Figure 1**). More specifically, four threshold methods will be used based on previously reported methodologies [**19-22**]:

- Fixed 41% threshold of the SUVmax within the respective lymphoma site,
- Fixed absolute SUV threshold of 2.5;
- SUVmax(lesion)/SUVmean liver >1.5
- Adaptative method: I(threshold)= [0.15 x I(mean)]+ I(background). I(mean) is calculated as the mean intensity of all pixels surrounded by the 70% Imax isocontour within the tumor; I(background) is defined as a SUVmean of liver [**23**].

After delineation of all individual lesions, patient MTV will be estimated as the sum of voxels with supra-threshold uptake, reported in ml, and TLG will be calculated as [MTV x SUVmean].

PET2 scans will be evaluated by visual analysis on the basis of Deauville-5-points-scale assigning Inadequate Response (IR) when at least one site shows FDG uptake higher than liver uptake (scores 4 and 5). Additionally, the variation of SUVmax, determined as the percentage reduction between the SUVmax in the tumor site with the most intense uptake on PET1 and the SUVmax in the tumor site with the most intense uptake on PET2 (ΔSUVmax) [**9**], will be computed. Similarly, will be calculated the variation of SUVmean, SUVpeak, MTV, and TLG, respectively.

Assessment of bulky masses and radiomics analyses

The definition of bulky masses will be determined as specified in EuroNet-PHL-C2 [**17**]. More specifically, a volume of a contiguous lymph node mass ≥ 200ml, measured by the three largest diameters on CT/MRI, will be considered as bulky. All bulky masses will be outlined using different threshold methods, as explained above, and analyzed on dedicated software for semi-quantitative and volumetric parameters. The same software will provide textural and shape features for radiomics analyses. The entire feature extraction will be performed using the freeware Local Image Features Extraction (LIFEx) software (http://www.lifexsoft.org) [**24,25**].

SUVmax will be defined as the maximum uptake in the segmented tumor. SUVmean will be measured as the average uptake in the tumor burden. SUVpeak will be computed as the average SUV in a 1ml region of tumor burden around the maximal SUV voxel. MTV will be the volume of the segmented tumor. TLG will be calculated as the product of SUVmean by MTV.

Among shape parameters, asphericity, convexity and 3D fractal dimensions will be computed [**15, 18, 26, 27**]. For the characterization of tumor texture, two methods will be used as previously reported [**11, 28, 29**]: analysis of the histogram of the voxel values within the tumor and the method accounting for the spatial arrangement of voxel values. By histogram-based method, on first-order

statistics, will be computed SD (standard deviation), Entropy, Energy, kurtosis and Skewness. To define the spatial arrangement of the voxel values within the tumor, four matrices will be computed from each VOI: gray-level co-occurance matrix (GLCM), neighborhood gray-level different matrix (NGLDM), gray-level zone length matrix (GLZLM) and gray-level run length matrix (GLRLM). All parameters obtainable by the software and possible limitations are better detailed at http://www.lifex soft.org [**24,25**].

Definition of morphological response

In pediatric HL patients presenting with morphological partial response on bulky masses and/or residual lymph nodes with largest diameter ≥ 2 cm, a multiparametric assessment (i.e. SUVmax, SUVmean, SUVpeak, MTV, TLG and texture analysis) will be performed. For this purpose, the International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017) will be used when necessary [**30**]. In particular, we will include in the analysis all cases with:

- Poor bulk response: < 50% volume reduction and/or at least one nodal site with largest diameter of ≥ 2 cm and non-assessable qPET-value due to brown fatty tissue [**17**].
- Partial Response: ≥30% decrease in the sum of longest diameters of target lesions but no complete response; positive PET (DS 4–5); any bone marrow involvement, no new lesions [**30**].
- Minor Response: ≥ 10% decrease in the sum of longest diameters of target lesions but not a partial response [**30**].

Sample size calculation

Given the limited number of robust data for volumetric and texture analysis in pediatric HL population, we considered adequate a sample size comprising all eligible patients. In the current study, we expect to enroll minimum 50-80 patients per year from the Italian Hodgkin Lymphoma Group out for the different AIEOP Italian Centers. Based on the data derived from the previous AIEOP-LH2004 trial, the estimated number of bulky masses is quoted around 50% of the enrolled cases. Consequently, the overall cohort to be included in the study is expected of round 400-500 patients, with half presenting with bulky masses, eligible for dedicated analyses.

PATIENT AND PUBLIC INVOLVEMENT

No patient involved

STATISTICAL ANALYSIS

Descriptive statistics will be performed using conventional metrics (mean, median, range). All metabolic and heterogeneity parameters will be correlated with each other and with the disease outcome and their diagnostic and prognostic role will be investigated. For continuous data, differences between groups will be compared by the T test or the Wilcoxon test, when appropriate. For rank correlation, we will use Spearman' correlation coefficient (rho). The different threshold methods used to outline all individual lesions will be compared by the Pearson correlation coefficient, linear regression, Bland-Altmann and logistic regression. Optimal cut-off values of the metabolic parameters and, in patients with bulky mass, also of textural/radiomics parameters for distinguishing inadequate response (IR) from adequate response (AR) to therapy will be defined by receiver operating characteristic (ROC) curves with respective areas under the curve (AUC). Patients with or without bulky mass will be divided into groups of complete metabolic response (CMR), partial metabolic response (PMR), no metabolic response (NMR) and progressive metabolic disease (PMD) and differences in metabolic and textural parameters will be investigated by analysis of variance (ANOVA). Linear regression will be applied to determine the relationship between response and all other variables. Statistical significance will be set for $p < 0.05$.

DISCUSSION

In literature there is evidence that metabolically active tumor volume determined by PET/CT is more advantageous than tumor volume measured by CT or MRI for predicting response to treatment in various malignancies afflicting both adult and the pediatric population. More specifically, in adult population, recent publications have demonstrated that the measurement of 3-dimensional disease volume (MTV) and metabolic activity (TLG) [**14**] can help predict outcomes in HL patients [**8, 9, 14, 30-32**]. This might suggest a similar implication also in pediatric HL, where the tumor volume may not change because of overlapping inflammatory processes correlated to therapy, while early changes of metabolic activity are most frequently reported.

Along with the above mentioned semi-quantitative parameters, it is possible to extract other quantitative features from PET-CT images, including intensity, heterogeneity, and shape within the tumor, potentially reflecting underlying biological characteristics [**14**]. These characteristics are embedded in the so called "Radiomics", a translational field of research aiming to extract high-dimensional data from clinical images to predict underlying biological characteristics of the disease [**33**]. Radiomic features are correlated to prognostic markers in cancer (i.e. hypoxia, angiogenesis, proliferation, etc.) and might be utilized for tumor response prediction and outcome prognostication. In pediatric population, especially in case of advanced stage disease, high dose therapeutic regimens represent the standard to cure, yet at the expense of early and delayed side

effects [**34**]. In this context, it becomes even more important to identify those factors capable of limiting the doses to the necessary therapeutic effect while reducing at maximum the undesirable consequences. These prerogatives have guided in the last decades clinical research in adult [**35, 36**] and pediatric HL [**6, 17**].

In the present study, we aim to identify prospectively the role of volumetric and texture (radiomic) characteristics better fulfilling the need for predictive and prognostic factors in pediatric HL. Thanks to a large sample size and to a preliminary methodological validation, we expect to obtain significant data on the added value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma.

LIST OF ABBREVIATIONS

AIEOP = Associazione Italiana di Ematologia e Oncologia Pediatrica

CT = computed tomography

DS = Deauville score

FDG = 18F-fluorodeoxyglucose

GLCM = gray-level co-occurance matrix

GLRLM = gray-level run length matrix

GLZLM = gray-level zone length matrix

HL = Hodgkin lymphoma

MRI = magnetic resonance imaging

MTV = metabolic tumor volume

NGLDM = neighborhood gray-level different matrix

OEPA = Vincristine Sulfate (Oncovin), Etoposide Phosphate, Prednisone. Doxorubicin Hydrochloride (Adriamycin)

PET = positron emission tomography

SD = standard deviation

SUV = standardized uptake value

TLG = total lesion glycolysis

VOI = volume of interest

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The current study has been approved by AIFA (Agenzia Italiana del Farmaco) the 9th of March 2018 (EudraCT 2012-004053-88, EM-04; AIFA/SC/P/27087 approved 09/03/2018). All procedures involving human participants will be performed in accordance with the ethical standards of the institutional and/or national research committee and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All patients will sign a written informed consent to participate in the study.

CONSENT FOR PUBBLICATION

Not applicable

AVAILABILIY OF DATA AND MATERIAL

Not applicable

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

AUTHORS' CONTRIBUTION

EL, CE, RB and MM planned, coordinated and conducted the study. Medical care is covered by the AIEOP Centers for the Hodgkin Lymphoma Study Group. Scientific program is planned by MM, EL, RB. Authors read and approved the final manuscript.

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REFERENCES

1. Cheson, B.D., et al. Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma: The Lugano Classification. *J Clin Oncol.* 2014, Vol. 32, 27, p. 3059-67.

2. Barrington, S.F., et al. Role of Imaging in the Staging and Response Assessment of Lymphoma: Consensus of International Conference on Malignant Lymphomas Imaging Working Group. *J Clin Oncol.* 2014, Vol. 32, 27, p. 3048-58.

3. Barrington, S.F., et al. FDG PET for therapy monitoring in Hodgkin and non-Hodgkin lymphoma. *Eur J Nucl Med Mol Imaging.* 2017, Vol. 44, p. 97-110.

4. Hasenclever D., et al. qPET – a quantitative extension of the Deauville scale to assess response in iterim FDG-PET scans in lymphoma. *Eur J Nucl Med Mol Imaging.* 2014, Vol. 41, p. 1301-8.

5. Mauz-Körholz C., et al. Procarbazine-free OEPA-COPDAC chemotherapy in boys and standard OPPA-COPP in girls have comparable effectiveness in pediatric Hodgkin's lymphoma: the GPOH-HD-2002 study. *J Clin Oncol.* 2016, Vol. 28, 23, p. 3680-6.

6. Rogasch JMM, et al. Pretherapeutic FDG-PET total metabolic tumor volume predicts response to induction therapy in pediatric Hodgkin's lymphoma. *BMC Cancer*. 2018; Vol. 18: p.521

7. Kostakoglu, L., et al. FDG-PET imaging for Hodgkin Lymphoma: Currente use and future applications. *Clin Adv Hematol Oncol.* 2014, Vol. 12, p. 20-35.

8. Meignan, M., et al. Metabolic tumou volumes measured at staging in lymphoma: Methodological evaluation on phantom experiments and patients. *Eur J Nucl Med Mol Imaging.* 2014, Vol. 41, p. 1113-1122.

9. Kanoun, S., et al. Baseline metabolic tumour volume is an indipendent prognostic factor in Hodgkin lymphoma. *Nucl Med Mol Imaging.* 2014, Vol. 41, p. 1735-1743.

10. Brooks, F.J., et al. Low-order non-spatial effects dominate second-order spatial effects in the texture quantifier analysis of 18F-FDG-PET images. *PLoS One.* 2015, Vol. 10, p. e0116574.

11. Buvat, I., et al. Tumor texture analysis in PET: Where do we stand? *J Nucl Med.* 2015, Vol. 56, p. 1642-44.

12. Kluge, R., et al. Current Role of FDG-PET in Pediatric Hodgkin's Lymphoma. *Semin Nucl Med.* 2017, Vol. 47, p. 242-257.

13. Ben Bouallegue, F. et al. Association between textural and morphological tumor indices on baseline PET-CT and early metabolic response on interim PET-CT in bulky malignant lymphomas. *Med Phys* 2017; Vol. 44, p. 4608–4619.

14. Milgrom, S.A., et al. A PET Radiomics Model to Predict Refractory Mediastinal Hodgkin Lymphoma.*Sci Rep*. 2019, Vol. 9, p. 1322.

15. Lopci, E., et al. Postchemotherapy PET evaluation correlates with patient outcome in paediatric Hodgkin's disease. *Eur J Nucl Med Mol Imaging.* 2011, Vol. 38, 9, p. 1620-7.

16. Lopci, E., et al. Additional role of FDG-PET in paediatric HD patients presenting with a partial response on CT: data deriving from a multicentric Italian study. *Eur J Nucl Med Mol Imaging.* 2012, Vol. 39, p. S155–S303.

17. Second International Inter-Group Study for Classical Hodgkin Lymphoma in Children and Adolescents. Final protocol version, 1st amendment. https://clinicaltrials.gov/ct2/show/NCT02684708.

18. Vanderhoek, M., et al. Impact of the Definition of Peak Standardized Uptake Value on Quantification of Treatment Response. *J Nucl Med.* 2012, Vol. 53, p. 4-11.

19. Boellaard, R., et al. FDG PET and PET/CT: EANM procedure guidelines for tumourPET imaging: version 1.0. *Eur J Nucl Med Mol Imaging.* 2010, Vol. 37, p. 181-200.

20. Ben Bouallègue, F., et al. Association between textural and morphological tumor indices on baseline PET-CT and early metabolic response on interim PET-CT in bulky malignant lymphomas. *Med Phys.* 2017, Vol. 44, p. 4608-19.

21. Mettler, J., et al. Metabolic Tumour Volume for Response Prediction in Advanced-Stage Hodgkin Lymphoma. *J Nucl Med.* 2018.

22. Cottereau, A.S., et al. Prognostic value of baseline metabolic tumor volume in early-stage Hodgkin lymphoma in the standard arm of the H10 trial. *Blood.* 2018, Vol. 131, p. 1456-63.

23. Nestle U., et al. Comparison of different methods for delineation of 18F-FDG PET-positive tissue for target volume definition in radiotherapy of patients with non-Small cell lung cancer. *J Nucl Med*. 2005; Vol. 46: p.1342-8.

24. Nioche. C., et al. A software for characterizing intra-tumor heterogeneity in multimodality imaging and establishing reference charts. *Eur J Nucl Med Mol Imaging*. 2016; Vol. 43: S156-S157.

25. Parvez, A., et al. 18F-FDG PET/CT metabolic tumor parameters and radiomics features in aggressive non-Hodgkin's lymphoma as predictors of treatment outcome and survival. *Ann Nucl Med*. 2018; Vol. 32,p. 410–416.

26. Castello, A., et al. Prognostic Impact of Intratumoral Heterogeneity Based on Fractal Geometry Analysis in Operated NSCLC Patients. *Mol Imaging Biol.* 2018, p. 965–972.

27. Grizzi, F., et al. The Complexity and Fractal Geometry of Nuclear Medicine Images. *Mol Imaging Biol.* 2018, p. 401–409.

28. Orlhac, F., et al. Tumor Texture Analysis in 18F-FDG PET: Relationships Between Texture Parameters, Histogram Indices, Standardized Uptake Values, Metabolic Volumes, and Total Lesion Glycolysis. *J Nucl Med.* 2014, Vol. 55, p. 414-422.

29. Ganeshan, B., et al. CT-based texture analysis potentially provides prognostic information complementary to interim fdg-pet for patients with hodgkin's and aggressive non-hodgkin's lymphomas. *Eur Radiol.* 2017, Vol. 27, p. 1012-1020.

30. Younes A. et al, International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017). *Ann Oncol*. 2017;Vol. 28: P. 1436-1447.

31. Song MK. et al., Metabolic tumor volume by positron emission tomography/computed tomography as a clinical parameter to determine therapeutic modality for early stage Hodgkin's lymphoma. *Cancer Sci* 2013; Vol. 104: p. 1656-61.

32. Akhtari M., et al. Reclassifying patients with early-stage Hodgkin lymphoma based on functional radiographic markers at presentation. *Blood.* 2018; Vol. 131: p. 84-94.

33. Rizzo S, et al. Radiomics: the facts and the challenges of image analysis. *Eur Radiol Exp* 2018;2:36.

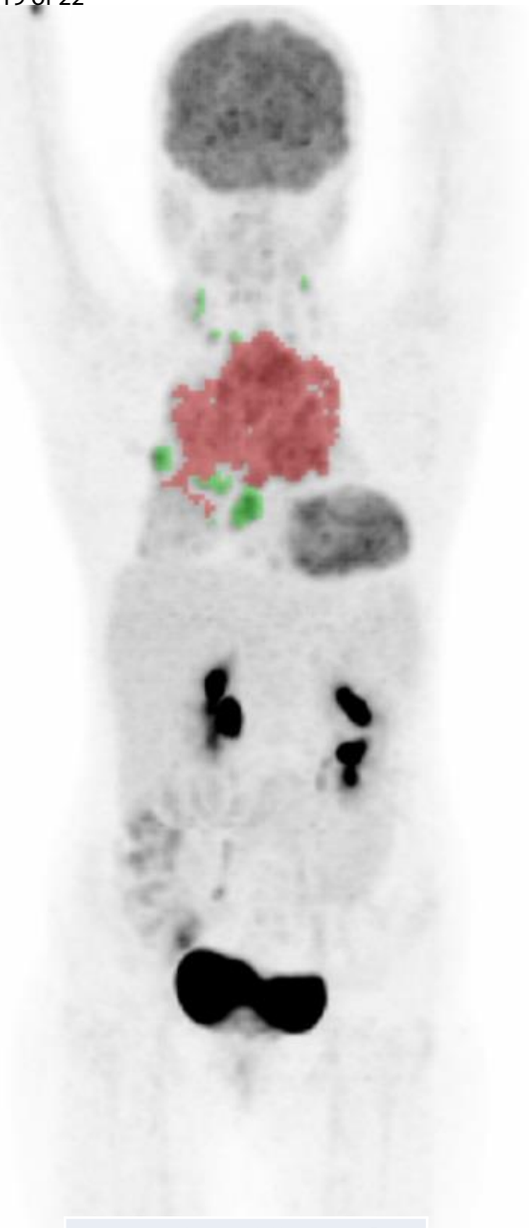
34. Lackner H., et al, Prospectiveevaluation of late effects after childhood cancer therapy with follow-up over 9 years. *Eur J Pediatr* 2000; Vol. 159: p. 750-758.

35. Borchmann P, et al. PET-guided treatment in patients with advanced-stage Hodgkin's lymphoma (HD18): final results of an open-label, international, randomised phase 3 trial by the German Hodgkin study group. *Lancet*. 2018;Vol. 390: p. 2790–802.

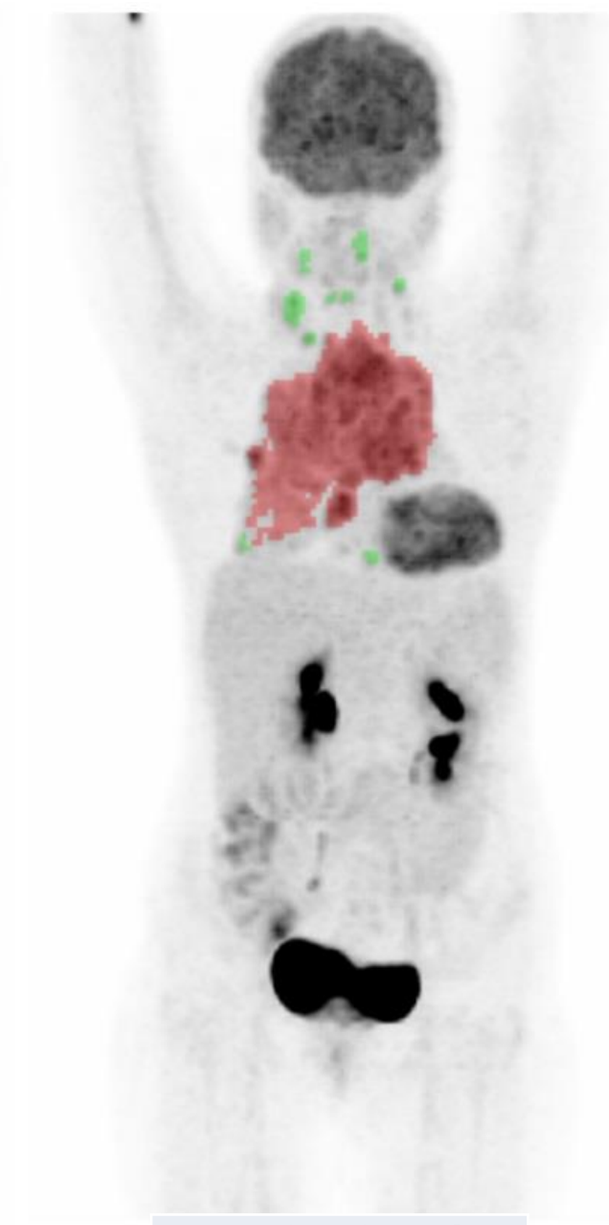
36. Casasnovas RO, et al. PET-adapted treatment for newly diagnosed advanced Hodgkin lymphoma (AHL2011): a randomised, multicentre, non-inferiority, phase 3 study. *Lancet Oncol* 2019; Vol. 20: p. 202-215.

FIGURE LEGEND

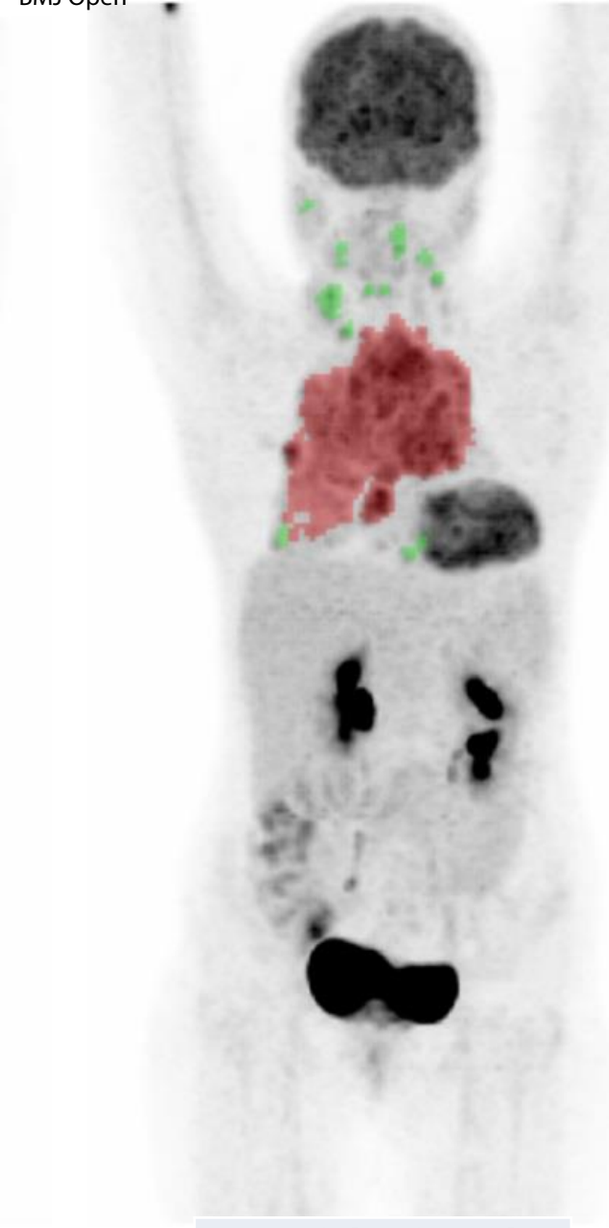
Figure 1: Comparative representation of the four segmentation techniques applied in our study protocol illustrated from left to right: fixed 41% threshold (V41%); fixed absolute SUV threshold of 2.5 (V2.5); SUVmax(lesion)/SUVmean liver >1.5 (Vliver); and adaptative method (AM). The same HL patient has been analyzed according to the above mentioned techniques and corresponding TMTV (total metabolic tumor volumes) at baseline have been displayed for comparison.



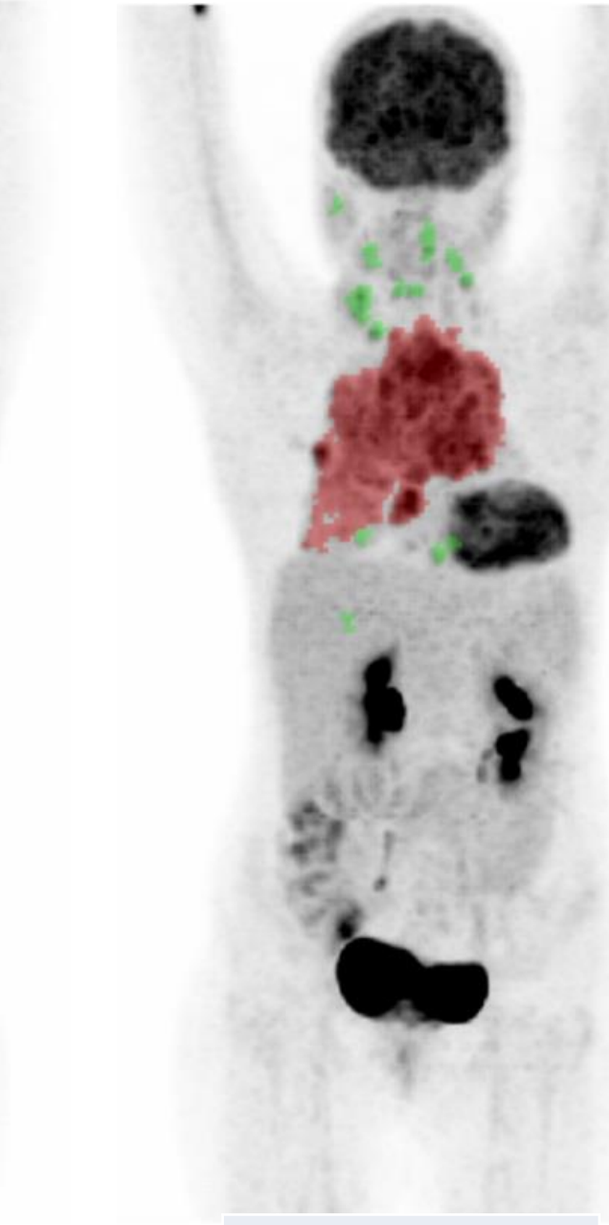
TMTV (V41%) Baseline
242,30



TMTV (V2.5) Baseline
471,00



TMTV (Vliver) Baseline
500,40



TMTV (AM) Baseline
538,3

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Additional value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma in the context of the euronet-PHL-C2-Trial.
Trial registration	2a	EudraCT 2012-004053-88, EM-04;
	2b	AIFA/SC/P/27087 approved 9th of March 2018
Protocol version	3	Amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Funding	4	Declarations, article page 10
Roles and responsibilities	5a	Responsible investigators: Egest Lopci, MD, PhD, Nuclear Medicine department, Humanitas Clinical and Research Hospital – IRCCS, Rozzano (MI), Italy Maurizio Mascarin, MD, AYA and Pediatric Radiotherapy IRCCS Centro di Riferimento Oncologico Roberta Burnelli, MD, Pediatric Onco-hematologic Unit, University Hospital S. Anna, Ferrara Caterina Elia, AYA and Pediatric Radiotherapy, IRCCS Centro di Riferimento Oncologico Arnoldo Piccardo, MD, Nuclear Medicine department, Galliera Hospital, Genoa, Italy. Eugenio Borsatti, MD, Nuclear Medicine department, Centro di Riferimento Oncologico, Aviano, Pordenone, Italy Pietro Zucchetta, MD, Nuclear Medicine Department, University Hospital, Padova, Italy Angelina Cistaro, MD, Positron Emission Tomography Centre, IRMET S.p.A. Affidea, Turin, Italy
	5b	Maurizio Mascarin, MD, AYA and Pediatric Radiotherapy IRCCS Centro di Riferimento Oncologico
	5c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
	5d	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Introduction		

Background and rationale	6a	Background, article page 4
	6b	Background, article page 4
Objectives	7	Study objectives, article page 4
Trial design	8	This is a prospective observational multicentric cohort study.

Methods: Participants, interventions, and outcomes

Study setting	9	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Eligibility criteria	10	Eligibility criteria, article page 5
Interventions	11a	N/A
	11b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
	11c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
	11d	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Outcomes	12	Study objectives, article page
Participant timeline	13	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Sample size	14	Sample size calculation, article page 7
Recruitment	15	AIEOP Hodgkin Lymphoma Study Group

Methods: Assignment of interventions (for controlled trials)

Allocation:		
Sequence generation	16a	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Allocation concealment mechanism	16b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Implementation	16c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Blinding (masking)	17a	Open label

	17b	N/A
Methods: Data collection, management, and analysis		
Data collection methods	18a	Statistical Analysis, article page 7
	18b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Data management	19	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Statistical methods	20a	Statistical Analysis, article page 7
	20b	Statistical Analysis, article page 7
	20c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group)
Methods: Monitoring		
Data monitoring	21a	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
	21b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Harms	22	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Auditing	23	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Ethics and dissemination		
Research ethics approval	24	The protocol has been already board (REC/IRB) approved
Protocol amendments	25	N/A
Consent or assent	26a	Local investigators affiliated to the AIEOP (Associazione Italiana Ematologia Oncologia Pediatrica) centers.
	26b	N/A
Confidentiality	27	As per EuroNet-PHL-C2 protocol and AIEOP policy
Declaration of interests	28	Declarations, article page 10
Access to data	29	As per EuroNet-PHL-C2 protocol and AIEOP policy

Ancillary and post-trial care	30	N/A
Dissemination policy	31a	Trial results will be communicated to participants, healthcare professionals, the public, and other relevant groups via scientific and congress publications.
	31b	Authorship eligibility will be provided to investigators based on their contribution to the study.
	31c	As per EuroNet-PHL-C2 protocol and AIEOP policy
Appendices		
Informed consent materials	32	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Biological specimens	33	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Additional value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma: an Italian multicentric study protocol.

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Additional value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma: an Italian multicentric study protocol.

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ABSTRACT

Introduction: Assessment of response to therapy in pediatric Hodgkin lymphoma (HL) patients by 18F-fluorodeoxyglucose PET/CT (FDG PET) has become a powerful tool for the discrimination of responders from non-responders. The addition of volumetric and texture analyses can be regarded as a valuable help for disease prognostication and biological characterization. Based on these premises, the AIEOP Hodgkin Lymphoma Study Group has designed a prospective evaluation of volumetric and texture analysis in the Italian cohort of patients enrolled in the EuroNet-PHL-C2.

Methods and Analysis: The primary objective is to compare volumetric assessment in HL patients at baseline and during the course of therapy with standard visual and semi-quantitative analyses. The secondary objective is to identify the impact of volumetric and texture analysis on bulky masses. The tertiary objective is to determine the additional value of multiparametric assessment in patients having a partial response on morphological imaging.

The overall cohort of the study is expected to be round 400-500 patients, with approximately half presenting with bulky masses. All PET scans of the Italian cohort will be analyzed for volumetric assessment, comprising metabolic tumor volume (MTV) and total lesion glycolysis (TLG) at baseline and during the course of therapy. A dedicated software will delineate semi-automatically contours using different threshold methods, and the impact of each segmentation techniques will be evaluated. Bulky will be defined on contiguous lymph node masses ≥200ml on CT/MRI. All bulky masses will be outlined and analyzed by the same software to provide textural features. Morphological assessment will be based in RECIL 2017 for response definition.

Ethics and Dissemination: The current study has been ethically approved (AIFA/SC/P/27087 approved 09/03/2018; EudraCT 2012-004053-88, EM-04). The results of the different analyses performed during and after study completion the will be actively disseminated through peer-reviewed journals, conference presentations, social media, print media and internet.

Keywords: FDG PET; Hodgkin's lymphoma; pediatric; volumetric analysis; response assessment; texture analysis; bulky masses; interim evaluation.

STRENGTHS AND LIMITATIONS OF THE STUDY

- This study will represent the largest analysis on volumetric and semi-quantitative parameters in pediatric HL undergoing a therapeutic trial.
- The dedicated evaluation of texture features in HL bulky masses, will allow for a solid definition of the impact of radiomics in this large pediatric population.
- Thanks to a comparative disease evaluation with both metabolic (PET) and morphological (CT/MRI) parameters, we will be able to assess the added value of the technique in pediatric HL patients presenting with a partial response to therapy.
- The segmentation used for volumetric analyses can be considered a limit, since the predefined threshold methods might not be applicable for all lesions, particularly during response assessment; hence, a preliminary validation study will be performed.
- Data extraction for radiomic features will be necessarily performed in PET exams obtained from different scanners and undergoing different reconstruction algorithms, although harmonization based on EANM guidelines is recommended for the trial.

INTRODUCTION

18F-fluorodeoxyglucose positron emission tomography (FDG PET) has become a standard diagnostic procedure for the assessment of response to therapy in adults and children with Hodgkin lymphoma. International guidelines recommend the using of Deauville five-point scale as a visual method for discriminating responders from non-responder patients [**1, 2**]. In 2014, the pediatric German group proposed the use of qPET with the intent to extend the Deauville score to a continuous scale and limit optical misinterpretation due to the influence of background activity [**3, 4**]. This quantitative method is being applied in the current EuroNet-PHL-C2 clinical trial, in which adapted therapy is based on quantitative FDG avidity of tumor masses on PET evaluation after 2 cycles of OEPA [**5, 6**]. This approach, however, postpones risk stratification at interim evaluation; therefore, the definition of imaging baseline predictors is highly desirable.

The implementation of metabolic tumor volume, as a sum of areas with an increased SUV inside the tumor, as well as the characterization of the heterogeneity of tumor metabolic patterns on FDG PET has become an emerging topic in nuclear medicine **[7**]. Several studies [**8-11**] have shown that the addition of volumetric and textural parameters can be a valuable help for disease prognostication and biological characterization of many tumor types, thus suggesting a similar implication for pediatric Hodgkin lymphoma [**12**]. While the concept of "Radiomics", consisting on the extraction of a large quantity of features from digital images via data-characterization algorithms has gained a proper place in predicting outcome and early metabolic response in adults with malignant lymphoma [**13, 14**] On the other hand, the scientific background and the results obtained from our previous studies in the context of the Italian AIEOP-LH2004 trial [**15, 16**] suggest an additional impact of FDG PET in patients with or without bulky disease presenting with residual masses on morphological evaluation with computed tomography (CT).

Given the abovementioned premises, the AIEOP Hodgkin Lymphoma Study Group has planned to perform in the Italian cohort of patients treated according to the EuroNet-PHL-C2 trial additional volumetric analyses to improve the evaluation of tumor burden computed at baseline FDG PET and to identify prognostic factors suitable for predicting early metabolic response to therapy in pediatric Hodgkin lymphoma (HL). In case of bulky disease, further textural and shape analysis in the baseline FDG PET will be performed to evaluate macroscopic and microscopic heterogeneity of tumor masses, as reflection of their aggressiveness and different chemotherapy sensitivity.

METHODS AND ANALYSIS

STUDY OBJECTIVES

This is a prospective observational multicentric cohort study. The primary objective of the study is to compare the diagnostic and prognostic role of volumetric assessment in HL patients at baseline and during the course of therapy with standard visual (Deauville score) and semi-quantitative (i.e. SUVmax, SUVmean, SUVpeak) analyses.

The secondary objective of the study is to identify the diagnostic and prognostic impact of texture analyses and the other metabolic parameters on bulky masses.

The tertiary objective of the study is to determine the additional predictive and prognostic value of multiparametric assessment (i.e. SUVmax, SUVmean, SUVpeak, MTV, TLG and texture analysis) in HL patients having a partial response on morphological imaging.

ELIGIBILITY CRITERIA

In accordance to the EuroNet-PHL-C2 trial, the population of our study will include pediatric patients of the Italian cohort, aged <25 years, with histologically confirmed primary diagnosis of classical Hodgkin's lymphoma, who will undergo FDG PET at baseline (PET1), after two cycles of induction OEPA therapy (PET2), and after the end of chemotherapy (PET3), in case of PET2 positive patients [**17**]. Patients will be stratified at baseline in one of the three Treatment Levels (TL) on basis of stage and risk factors, confirmed by central review: TL-1, TL-2, TL-3 for low, intermediate and advanced HL, respectively [**6, 17**].

STUDY TIMELINE

The protocol herein illustrated represents a parallel study on PET imaging performed after the EuroNet-PHL-C2 trial amendment (Amendment Nr. 04, dated 2017-07-31) on the Italian cohort of patients. The study has been also submitted and approved by the Italian authority (AIFA) the date 2018-03-09. Consequently, the timeline of the protocol will be as follows: I) enrollment period will start from the 10th of March 2018 until 31st of December 2020; II) follow-up period will last 5 years after last enrolment day; III) study completion is planned before 31st December 2025.

METHODOLOGY

Whole body assessment of HL

All FDG PET scans performed in the Italian cohort of patients undergoing the EuroNet-PHL-C2 trail will be analyzed with additional volumetric assessment comprising metabolic tumor volume (MTV) and total lesion glycolysis (TLG) at baseline and during the course of therapy.

In each patient, HL lesions will be identified by visual analysis and corresponding SUVmax, SUVmean and SUVpeak [**18**] will be determined as the pixel with the highest value of uptake, the

mean value of uptake and the average value of uptake in a VOI (volume of interest) of 1ml that surrounds the voxel with the highest activity, respectively. A dedicated software will be used to delineate, semi-automatically, contours of the lesions using different threshold methods and the impact of segmentation technique will be evaluated (**Figure 1**). More specifically, four threshold methods will be used based on previously reported methodologies [**19-22**]:

- Fixed 41% threshold of the SUVmax within the respective lymphoma site,
- Fixed absolute SUV threshold of 2.5;
- SUVmax(lesion)/SUVmean liver >1.5
- Adaptative method: I(threshold)= [0.15 x I(mean)]+ I(background). I(mean) is calculated as the mean intensity of all pixels surrounded by the 70% Imax isocontour within the tumor; I(background) is defined as a SUVmean of liver [**23**].

After delineation of all individual lesions, patient MTV will be estimated as the sum of voxels with supra-threshold uptake, reported in ml, and TLG will be calculated as [MTV x SUVmean].

PET2 scans will be evaluated by visual analysis on the basis of Deauville-5-points-scale assigning Inadequate Response (IR) when at least one site shows FDG uptake higher than liver uptake (scores 4 and 5). Additionally, the variation of SUVmax, determined as the percentage reduction between the SUVmax in the tumor site with the most intense uptake on PET1 and the SUVmax in the tumor site with the most intense uptake on PET2 (ΔSUVmax) [**9**], will be computed. Similarly, will be calculated the variation of SUVmean, SUVpeak, MTV, and TLG, respectively.

Assessment of bulky masses and radiomics analyses

The definition of bulky masses will be determined as specified in EuroNet-PHL-C2 [**17**]. More specifically, a volume of a contiguous lymph node mass ≥ 200ml, measured by the three largest diameters on CT/MRI, will be considered as bulky. All bulky masses will be outlined using different threshold methods, as explained above, and analyzed on dedicated software for semi-quantitative and volumetric parameters. The same software will provide textural and shape features for radiomics analyses. The entire feature extraction will be performed using the freeware Local Image Features Extraction (LIFEx) software (http://www.lifexsoft.org) [**24,25**].

SUVmax will be defined as the maximum uptake in the segmented tumor. SUVmean will be measured as the average uptake in the tumor burden. SUVpeak will be computed as the average SUV in a 1ml region of tumor burden around the maximal SUV voxel. MTV will be the volume of the segmented tumor. TLG will be calculated as the product of SUVmean by MTV.

Among shape parameters, asphericity, convexity and 3D fractal dimensions will be computed [**15, 18, 26, 27**]. For the characterization of tumor texture, two methods will be used as previously

reported [**11, 28, 29**]: analysis of the histogram of the voxel values within the tumor and the method accounting for the spatial arrangement of voxel values. By histogram-based method, on first-order statistics, will be computed SD (standard deviation), Entropy, Energy, kurtosis and Skewness. To define the spatial arrangement of the voxel values within the tumor, four matrices will be computed from each VOI: gray-level co-occurance matrix (GLCM), neighborhood gray-level different matrix (NGLDM), gray-level zone length matrix (GLZLM) and gray-level run length matrix (GLRLM). All parameters obtainable by the software and possible limitations are better detailed at http://www.lifex soft.org [**24,25**].

Definition of morphological response

In pediatric HL patients presenting with morphological partial response on bulky masses and/or residual lymph nodes with largest diameter ≥ 2 cm, a multiparametric assessment (i.e. SUVmax, SUVmean, SUVpeak, MTV, TLG and texture analysis) will be performed. For this purpose, the International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017) will be used when necessary [**30**]. In particular, we will include in the analysis all cases with:

- Poor bulk response: < 50% volume reduction and/or at least one nodal site with largest diameter of ≥ 2 cm and non-assessable qPET-value due to brown fatty tissue [**17**].
- Partial Response: ≥30% decrease in the sum of longest diameters of target lesions but no complete response; positive PET (DS 4–5); any bone marrow involvement, no new lesions [**30**].
- Minor Response: ≥ 10% decrease in the sum of longest diameters of target lesions but not a partial response [**30**].

Sample size calculation

Given the limited number of robust data for volumetric and texture analysis in pediatric HL population, we considered adequate a sample size comprising all eligible patients. In the current study, we expect to enroll minimum 50-80 patients per year from the Italian Hodgkin Lymphoma Group out for the different AIEOP Italian Centers. Based on the data derived from the previous AIEOP-LH2004 trial, the estimated number of bulky masses is quoted around 50% of the enrolled cases. Consequently, the overall cohort to be included in the study is expected of round 400-500 patients, with half presenting with bulky masses, eligible for dedicated analyses.

PATIENT AND PUBLIC INVOLVEMENT

No patient involved

STATISTICAL ANALYSIS

Descriptive statistics will be performed using conventional metrics (mean, median, range). All metabolic and heterogeneity parameters will be correlated with each other and with the disease outcome and their diagnostic and prognostic role will be investigated. For continuous data, differences between groups will be compared by the T test or the Wilcoxon test, when appropriate. For rank correlation, we will use Spearman' correlation coefficient (rho). The different threshold methods used to outline all individual lesions will be compared by the Pearson correlation coefficient, linear regression, Bland-Altmann and logistic regression. Optimal cut-off values of the metabolic parameters and, in patients with bulky mass, also of textural/radiomics parameters for distinguishing inadequate response (IR) from adequate response (AR) to therapy will be defined by receiver operating characteristic (ROC) curves with respective areas under the curve (AUC). Patients with or without bulky mass will be divided into groups of complete metabolic response (CMR), partial metabolic response (PMR), no metabolic response (NMR) and progressive metabolic disease (PMD) and differences in metabolic and textural parameters will be investigated by analysis of variance (ANOVA). Linear regression will be applied to determine the relationship between response and all other variables. Statistical significance will be set for $p < 0.05$.

DISCUSSION

In literature there is evidence that metabolically active tumor volume determined by PET/CT is more advantageous than tumor volume measured by CT or MRI for predicting response to treatment in various malignancies afflicting both adult and the pediatric population. More specifically, in adult population, recent publications have demonstrated that the measurement of 3-dimensional disease volume (MTV) and metabolic activity (TLG) [**14**] can help predict outcomes in HL patients [**8, 9, 14, 30-32**]. This might suggest a similar implication also in pediatric HL, where the tumor volume may not change because of overlapping inflammatory processes correlated to therapy, while early changes of metabolic activity are most frequently reported.

Along with the above mentioned semi-quantitative parameters, it is possible to extract other quantitative features from PET-CT images, including intensity, heterogeneity, and shape within the tumor, potentially reflecting underlying biological characteristics [**14**]. These characteristics are embedded in the so called "Radiomics", a translational field of research aiming to extract high-dimensional data from clinical images to predict underlying biological characteristics of the disease [**33**]. Radiomic features are correlated to prognostic markers in cancer (i.e. hypoxia, angiogenesis, proliferation, etc.) and might be utilized for tumor response prediction and outcome prognostication. In pediatric population, especially in case of advanced stage disease, high dose

therapeutic regimens represent the standard to cure, yet at the expense of early and delayed side effects [**34**]. In this context, it becomes even more important to identify those factors capable of limiting the doses to the necessary therapeutic effect while reducing at maximum the undesirable consequences. These prerogatives have guided in the last decades clinical research in adult [**35, 36**] and pediatric HL [**6, 17**].

In the present study, we aim to identify prospectively the role of volumetric and texture (radiomic) characteristics better fulfilling the need for predictive and prognostic factors in pediatric HL. Thanks to a large sample size and to a preliminary methodological validation, we expect to obtain significant data on the added value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma.

ETHICS AND DISSEMINATION:

The current study has been approved by AIFA (Agenzia Italiana del Farmaco) the 9th of March 2018 (EudraCT 2012-004053-88, EM-04; AIFA/SC/P/27087 approved 09/03/2018). All procedures involving human participants will be performed in accordance with the ethical standards of the institutional and/or national research committee and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All patients will sign a written informed consent to participate in the study. The results of the different analyses performed during and after study completion the will be actively disseminated through peer-reviewed journals, conference presentations, social media, print media and internet.

REFERENCES

1. Cheson, B.D., et al. Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma: The Lugano Classification. *J Clin Oncol.* 2014, Vol. 32, 27, p. 3059-67.

2. Barrington, S.F., et al. Role of Imaging in the Staging and Response Assessment of Lymphoma: Consensus of International Conference on Malignant Lymphomas Imaging Working Group. *J Clin Oncol.* 2014, Vol. 32, 27, p. 3048-58.

3. Barrington, S.F., et al. FDG PET for therapy monitoring in Hodgkin and non-Hodgkin lymphoma. *Eur J Nucl Med Mol Imaging.* 2017, Vol. 44, p. 97-110.

4. Hasenclever D., et al. qPET – a quantitative extension of the Deauville scale to assess response in iterim FDG-PET scans in lymphoma. *Eur J Nucl Med Mol Imaging.* 2014, Vol. 41, p. 1301-8.

5. Mauz-Körholz C., et al. Procarbazine-free OEPA-COPDAC chemotherapy in boys and standard OPPA-COPP in girls have comparable effectiveness in pediatric Hodgkin's lymphoma: the GPOH-HD-2002 study. *J Clin Oncol.* 2016, Vol. 28, 23, p. 3680-6.

6. Rogasch JMM, et al. Pretherapeutic FDG-PET total metabolic tumor volume predicts response to induction therapy in pediatric Hodgkin's lymphoma. *BMC Cancer*. 2018; Vol. 18: p.521

7. Kostakoglu, L., et al. FDG-PET imaging for Hodgkin Lymphoma: Currente use and future applications. *Clin Adv Hematol Oncol.* 2014, Vol. 12, p. 20-35.

8. Meignan, M., et al. Metabolic tumou volumes measured at staging in lymphoma: Methodological evaluation on phantom experiments and patients. *Eur J Nucl Med Mol Imaging.* 2014, Vol. 41, p. 1113-1122.

9. Kanoun, S., et al. Baseline metabolic tumour volume is an indipendent prognostic factor in Hodgkin lymphoma. *Nucl Med Mol Imaging.* 2014, Vol. 41, p. 1735-1743.

10. Brooks, F.J., et al. Low-order non-spatial effects dominate second-order spatial effects in the texture quantifier analysis of 18F-FDG-PET images. *PLoS One.* 2015, Vol. 10, p. e0116574.

11. Buvat, I., et al. Tumor texture analysis in PET: Where do we stand? *J Nucl Med.* 2015, Vol. 56, p. 1642-44.

12. Kluge, R., et al. Current Role of FDG-PET in Pediatric Hodgkin's Lymphoma. *Semin Nucl Med.* 2017, Vol. 47, p. 242-257.

13. Ben Bouallegue, F. et al. Association between textural and morphological tumor indices on baseline PET-CT and early metabolic response on interim PET-CT in bulky malignant lymphomas. *Med Phys* 2017; Vol. 44, p. 4608–4619.

14. Milgrom, S.A., et al. A PET Radiomics Model to Predict Refractory Mediastinal Hodgkin Lymphoma.*Sci Rep*. 2019, Vol. 9, p. 1322.

15. Lopci, E., et al. Postchemotherapy PET evaluation correlates with patient outcome in paediatric Hodgkin's disease. *Eur J Nucl Med Mol Imaging.* 2011, Vol. 38, 9, p. 1620-7.

16. Lopci, E., et al. Additional role of FDG-PET in paediatric HD patients presenting with a partial response on CT: data deriving from a multicentric Italian study. *Eur J Nucl Med Mol Imaging.* 2012, Vol. 39, p. S155–S303.

17. Second International Inter-Group Study for Classical Hodgkin Lymphoma in Children and Adolescents. Final protocol version, 1st amendment. https://clinicaltrials.gov/ct2/show/NCT02684708.

18. Vanderhoek, M., et al. Impact of the Definition of Peak Standardized Uptake Value on Quantification of Treatment Response. *J Nucl Med.* 2012, Vol. 53, p. 4-11.

19. Boellaard, R., et al. FDG PET and PET/CT: EANM procedure guidelines for tumourPET imaging: version 1.0. *Eur J Nucl Med Mol Imaging.* 2010, Vol. 37, p. 181-200.

20. Ben Bouallègue, F., et al. Association between textural and morphological tumor indices on baseline PET-CT and early metabolic response on interim PET-CT in bulky malignant lymphomas. *Med Phys.* 2017, Vol. 44, p. 4608-19.

21. Mettler, J., et al. Metabolic Tumour Volume for Response Prediction in Advanced-Stage Hodgkin Lymphoma. *J Nucl Med.* 2018.

22. Cottereau, A.S., et al. Prognostic value of baseline metabolic tumor volume in early-stage Hodgkin lymphoma in the standard arm of the H10 trial. *Blood.* 2018, Vol. 131, p. 1456-63.

23. Nestle U., et al. Comparison of different methods for delineation of 18F-FDG PET-positive tissue for target volume definition in radiotherapy of patients with non-Small cell lung cancer. *J Nucl Med*. 2005; Vol. 46: p.1342-8.

24. Nioche. C., et al. A software for characterizing intra-tumor heterogeneity in multimodality imaging and establishing reference charts. *Eur J Nucl Med Mol Imaging*. 2016; Vol. 43: S156-S157.

25. Parvez, A., et al. 18F-FDG PET/CT metabolic tumor parameters and radiomics features in aggressive non-Hodgkin's lymphoma as predictors of treatment outcome and survival. *Ann Nucl Med*. 2018; Vol. 32,p. 410–416.

26. Castello, A., et al. Prognostic Impact of Intratumoral Heterogeneity Based on Fractal Geometry Analysis in Operated NSCLC Patients. *Mol Imaging Biol.* 2018, p. 965–972.

27. Grizzi, F., et al. The Complexity and Fractal Geometry of Nuclear Medicine Images. *Mol Imaging Biol.* 2018, p. 401–409.

28. Orlhac, F., et al. Tumor Texture Analysis in 18F-FDG PET: Relationships Between Texture Parameters, Histogram Indices, Standardized Uptake Values, Metabolic Volumes, and Total Lesion Glycolysis. *J Nucl Med.* 2014, Vol. 55, p. 414-422.

29. Ganeshan, B., et al. CT-based texture analysis potentially provides prognostic information complementary to interim fdg-pet for patients with hodgkin's and aggressive non-hodgkin's lymphomas. *Eur Radiol.* 2017, Vol. 27, p. 1012-1020.

30. Younes A. et al, International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017). *Ann Oncol*. 2017;Vol. 28: P. 1436-1447.

31. Song MK. et al., Metabolic tumor volume by positron emission tomography/computed tomography as a clinical parameter to determine therapeutic modality for early stage Hodgkin's lymphoma. *Cancer Sci* 2013; Vol. 104: p. 1656-61.

32. Akhtari M., et al. Reclassifying patients with early-stage Hodgkin lymphoma based on functional radiographic markers at presentation. *Blood.* 2018; Vol. 131: p. 84-94.

33. Rizzo S, et al. Radiomics: the facts and the challenges of image analysis. *Eur Radiol Exp* 2018;2:36.

34. Lackner H., et al, Prospectiveevaluation of late effects after childhood cancer therapy with follow-up over 9 years. *Eur J Pediatr* 2000; Vol. 159: p. 750-758.

35. Borchmann P, et al. PET-guided treatment in patients with advanced-stage Hodgkin's lymphoma (HD18): final results of an open-label, international, randomised phase 3 trial by the German Hodgkin study group. *Lancet*. 2018;Vol. 390: p. 2790–802.

36. Casasnovas RO, et al. PET-adapted treatment for newly diagnosed advanced Hodgkin lymphoma (AHL2011): a randomised, multicentre, non-inferiority, phase 3 study. *Lancet Oncol* 2019; Vol. 20: p. 202-215.

AUTHORS' CONTRIBUTION

Egesta Lopci contributed in planning, conception, design, study coordination and conduct, data acquisition, analysis, interpretation, and reporting of the work in the current paper; Caterina Elia contributed in planning, study coordination and conduct, data collection and final approval of manuscript; Roberta Burnelli contributed in planning, study coordination and conduct, data collection and final approval of manuscript; Arnoldo Piccardo, Angelo Castello, Eugenio Borsatti, Pietro Zucchetta and Angelina Cistaro contributed in study conduct, data collection and final approval of manuscript; Maurizio Mascarin contributed in planning, conception, design, study coordination and conduct, data collection and final approval of manuscript. AIEOP Centers for the Hodgkin Lymphoma Study Group contribute for the medical care of all study participants.

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

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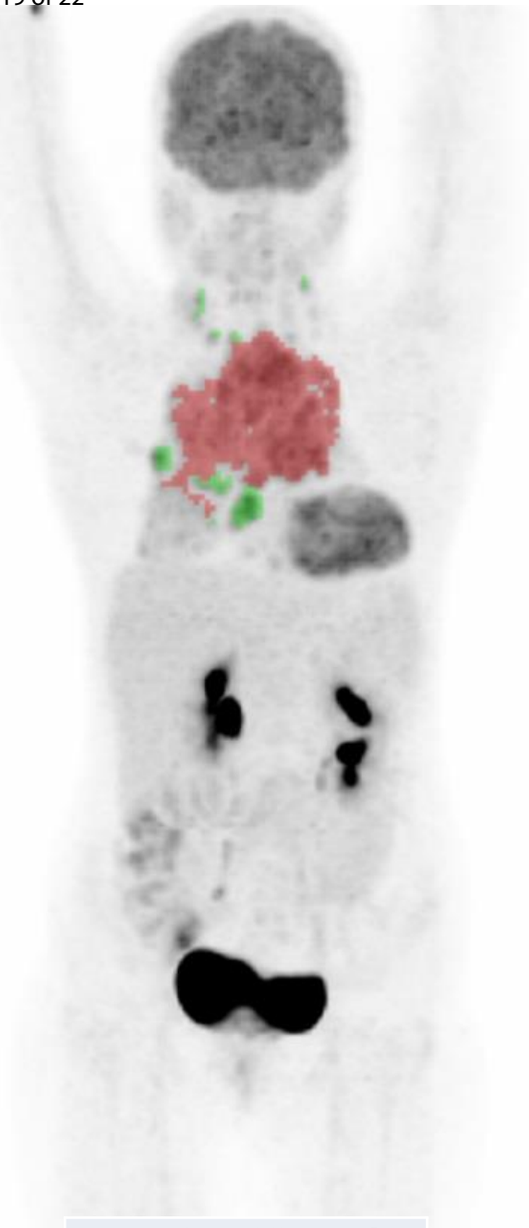
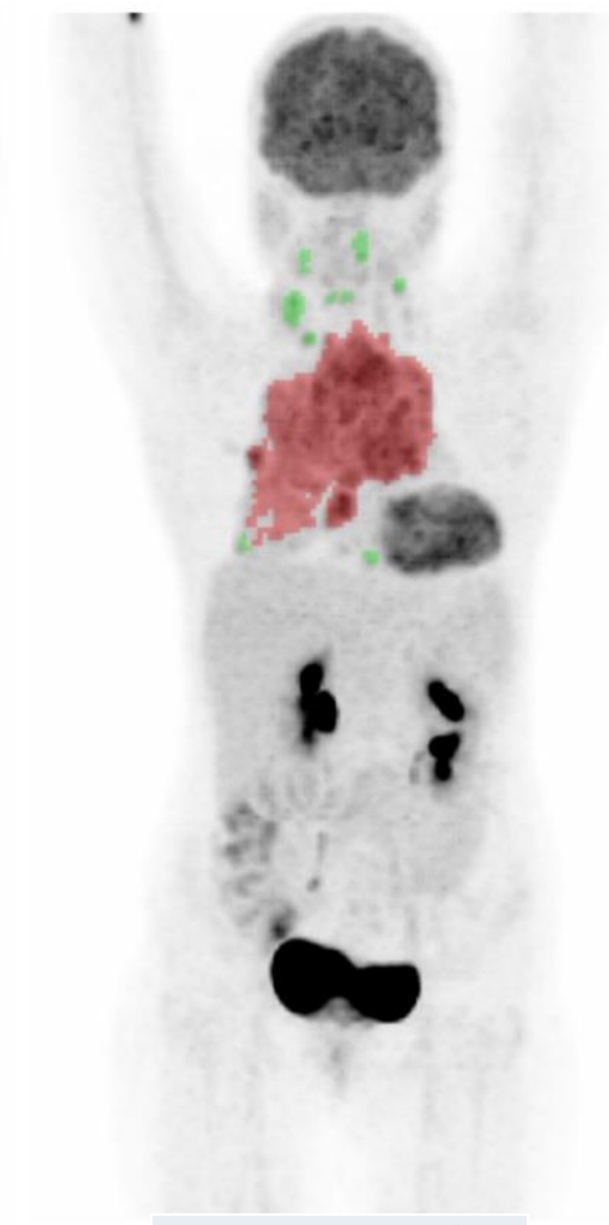
FIGURE LEGEND

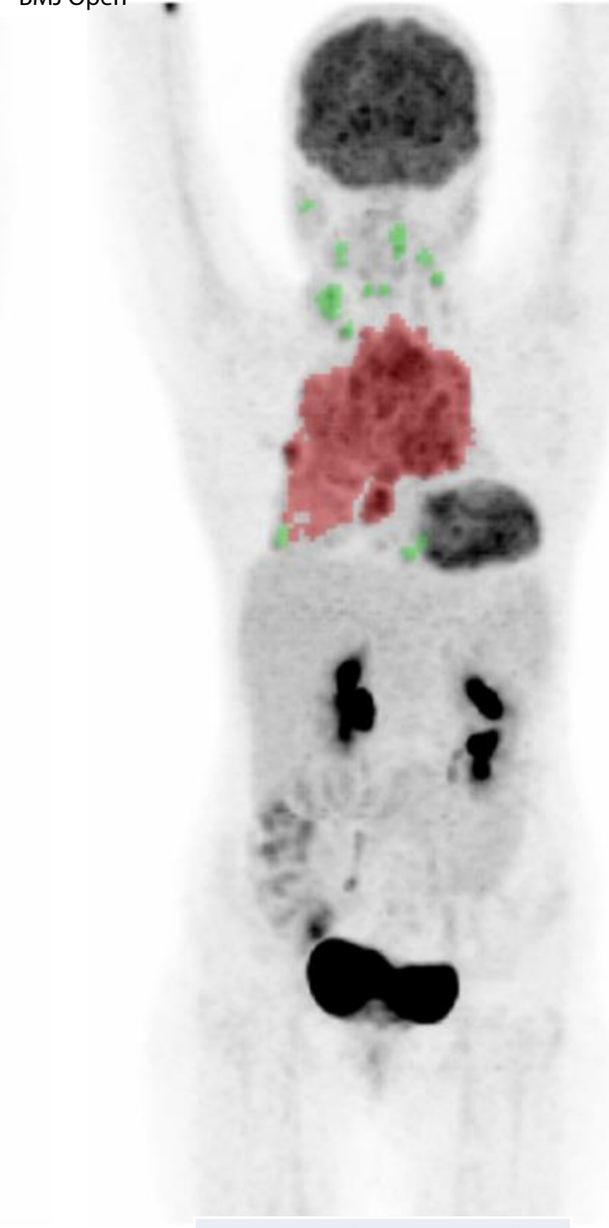
Figure 1: Comparative representation of the four segmentation techniques applied in our study protocol illustrated from left to right: fixed 41% threshold (V41%); fixed absolute SUV threshold of 2.5 (V2.5); SUVmax(lesion)/SUVmean liver >1.5 (Vliver); and adaptative method (AM). The same HL patient has been analyzed according to the above mentioned techniques and corresponding TMTV (total metabolic tumor volumes) at baseline have been displayed for comparison.



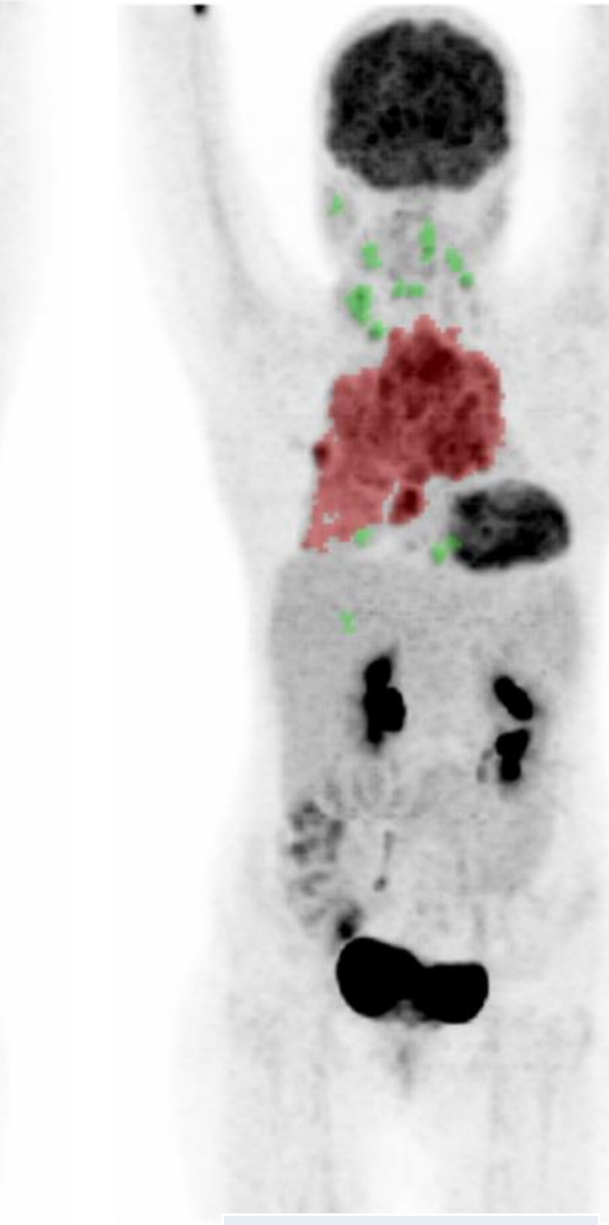
TMTV (V41%) Baseline
242,30



TMTV (V2.5) Baseline
471,00



TMTV (Vliver) Baseline
500,40



TMTV (AM) Baseline
538,3

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Additional value of volumetric and texture analysis on FDG PET assessment in pediatric Hodgkin Lymphoma in the context of the euronet-PHL-C2-Trial.
Trial registration	2a	EudraCT 2012-004053-88, EM-04;
	2b	AIFA/SC/P/27087 approved 9th of March 2018
Protocol version	3	Amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Funding	4	Declarations, article page 10
Roles and responsibilities	5a	Responsible investigators: Egest Lopci, MD, PhD, Nuclear Medicine, IRCCS - Humanitas Research Hospital, Rozzano (MI), Italy Maurizio Mascarin, MD, AYA and Pediatric Radiotherapy IRCCS Centro di Riferimento Oncologico Roberta Burnelli, MD, Pediatric Onco-hematologic Unit, University Hospital S. Anna, Ferrara Caterina Elia, AYA and Pediatric Radiotherapy, IRCCS Centro di Riferimento Oncologico Arnoldo Piccardo, MD, Nuclear Medicine department, Galliera Hospital, Genoa, Italy. Eugenio Borsatti, MD, Nuclear Medicine department, Centro di Riferimento Oncologico, Aviano, Pordenone, Italy Pietro Zucchetta, MD, Nuclear Medicine Department, University Hospital, Padova, Italy Angelina Cistaro, MD, Positron Emission Tomography Centre, IRMET S.p.A. Affidea, Turin, Italy
	5b	Maurizio Mascarin, MD, AYA and Pediatric Radiotherapy IRCCS Centro di Riferimento Oncologico
	5c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
	5d	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Introduction		

Background and rationale	6a	Background, article page 4
	6b	Background, article page 4
Objectives	7	Study objectives, article page 4
Trial design	8	This is a prospective observational multicentric cohort study.

Methods: Participants, interventions, and outcomes

Study setting	9	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Eligibility criteria	10	Eligibility criteria, article page 5
Interventions	11a	N/A
	11b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
	11c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
	11d	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Outcomes	12	Study objectives, article page
Participant timeline	13	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Sample size	14	Sample size calculation, article page 7
Recruitment	15	AIEOP Hodgkin Lymphoma Study Group

Methods: Assignment of interventions (for controlled trials)

Allocation:		
Sequence generation	16a	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Allocation concealment mechanism	16b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Implementation	16c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Blinding (masking)	17a	Open label

	17b	N/A
Methods: Data collection, management, and analysis		
Data collection methods	18a	Statistical Analysis, article page 7
	18b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Data management	19	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Statistical methods	20a	Statistical Analysis, article page 7
	20b	Statistical Analysis, article page 7
	20c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group)
Methods: Monitoring		
Data monitoring	21a	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
	21b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Harms	22	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Auditing	23	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31 and AIEOP Hodgkin Lymphoma Study Group
Ethics and dissemination		
Research ethics approval	24	The protocol has been already board (REC/IRB) approved
Protocol amendments	25	N/A
Consent or assent	26a	Local investigators affiliated to the AIEOP (Associazione Italiana Ematologia Oncologia Pediatrica) centers.
	26b	N/A
Confidentiality	27	As per EuroNet-PHL-C2 protocol and AIEOP policy
Declaration of interests	28	Declarations, article page 10
Access to data	29	As per EuroNet-PHL-C2 protocol and AIEOP policy

Ancillary and post-trial care	30	N/A
Dissemination policy	31a	Trial results will be communicated to participants, healthcare professionals, the public, and other relevant groups via scientific and congress publications.
	31b	Authorship eligibility will be provided to investigators based on their contribution to the study.
	31c	As per EuroNet-PHL-C2 protocol and AIEOP policy
Appendices		
Informed consent materials	32	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
Biological specimens	33	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.