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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.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-041252
Article Type:	Protocol
Date Submitted by the Author:	10-Jun-2020
Complete List of Authors:	Lopci, Egesta; Istituto Clinico Humanitas Burnelli, Roberta Elia, Caterina Piccardo, Arnoldo; Ente Ospedaliero Ospedali Galliera, Castello, Angelo Borsatti, Eugenio Zucchetta, Pietro Cistaro, Angelina Mascarin, Maurizio
Keywords:	Lymphoma < HAEMATOLOGY, Nuclear radiology < RADIOLOGY & IMAGING, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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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.

13 Egesta Lopci (1), Roberta Burnelli (2), Caterina Elia (3), Arnoldo Piccardo (4), Angelo
14 Castello (1), Eugenio Borsatti (5), Pietro Zucchetta (6), Angelina Cistaro (4), Maurizio
15 Mascarin (3) and AIEOP Hodgkin Lymphoma Study Group.
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1. Nuclear Medicine department, Humanitas Clinical and Research Hospital – IRCCS, Via Manzoni 56, 20089 Rozzano (MI), Italy;
 2. Pediatric Onco-hematologic Unit, University Hospital S. Anna, Ferrara, Italy.
 3. AYA and Pediatric Radiotherapy, IRCCS Centro di Riferimento Oncologico, Aviano, PN,
 4. Nuclear Medicine, Galliera Hospital, Genoa, Italy.
 5. Nuclear Medicine, Centro di Riferimento Oncologico, Aviano, Pordenone, Italy
 6. Nuclear Medicine Department, University Hospital, Padova, Italy.

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Corresponding author:

Egest Lopci, MD, PhD

Nuclear Medicine department

Humanitas Clinical and Research Hospital – IRCCS

Via Manzoni 56, CAP 20089, Rozzano (MI), Italy

Email: egesta.lopci@gmail.com

egesta.lopci@cancercenter.humanitas.it

Roberta Burnelli, MD

Pediatric Onco-hematologic Unit

University Hospital S. Anna, Ferrara

Email: roberta.burnelli@unife.it

Caterina Elia

AYA and Pediatric Radiotherapy

1
2
3 IRCCS Centro di Riferimento Oncologico

4 Email: eliacaterina@libero.it;
5
6
7

8 Arnaldo Piccardo, MD

9 Nuclear Medicine department

10 Galliera Hospital, Genoa, Italy.

11 Email: arnaldo.piccardo@galliera.it
12
13
14
15
16

17 Angelo Castello, MD

18 Nuclear Medicine department

19 Humanitas Clinical and Research Hospital – IRCCS

20 Via Manzoni 56, CAP 20089, Rozzano (MI), Italy

21 Email: angelo.castello@humanitas.it
22
23
24
25
26

27 Eugenio Borsatti, MD

28 Nuclear Medicine department

29 Centro di Riferimento Oncologico, Aviano, Pordenone, Italy

30 Email: eborsatti@cro.it
31
32
33
34
35

36 Pietro Zucchetta, MD

37 Nuclear Medicine Department

38 University Hospital, Padova, Italy

39 Email: pietro.zucchetta@unipd.it
40
41
42
43
44

45 Angelina Cistaro, MD

46 Nuclear Medicine department

47 Galliera Hospital, Genoa, Italy.

48 Email: angelina.cistaro@galliera.it
49
50
51
52

53 Maurizio Mascarin, MD

54 AYA and Pediatric Radiotherapy

55 IRCCS Centro di Riferimento Oncologico

56 Email: phl.italy@cro.it; mascarin@cro.it
57
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59
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ABSTRACT

Introduction: Assessment of response to therapy in pediatric Hodgkin lymphoma (HL) patients by ^{18}F -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 $\geq 200\text{ml}$ 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. SUV_{max}, SUV_{mean}, SUV_{peak}) analyses.

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

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

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

30 **Whole body assessment of HL**

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32 All FDG PET scans performed in the Italian cohort of patients undergoing the EuroNet-PHL-C2
33 trail will be analyzed with additional volumetric assessment comprising metabolic tumor volume
34 (MTV) and total lesion glycolysis (TLG) at baseline and during the course of therapy.
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37 In each patient, HL lesions will be identified by visual analysis and corresponding SUVmax,
38 SUVmean and SUVpeak [16] will be determined as the pixel with the highest value of uptake, the
39 mean value of uptake and the average value of uptake in a VOI (volume of interest) of 1ml that
40 surrounds the voxel with the highest activity, respectively. A dedicated software will be used to
41 delineate, semi-automatically, contours of the lesions using different threshold methods and the
42 impact of segmentation technique will be evaluated (Figure 1). More specifically, four threshold
43 methods will be used based on previously reported methodologies [17-20]:
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- 49 • Fixed 41% threshold of the SUVmax within the respective lymphoma site,
- 50 • Fixed absolute SUV threshold of 2.5;
- 51 • SUVmax(lesion)/SUVmean liver >1.5
- 52 • Adaptative method: $I(\text{threshold}) = [0.15 \times I(\text{mean})] + I(\text{background})$. $I(\text{mean})$ is calculated as the
53 mean intensity of all pixels surrounded by the 70% I_{max} isocontour within the tumor;
54 $I(\text{background})$ is defined as a SUVmean of liver [21].
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3 After delineation of all individual lesions, patient MTV will be estimated as the sum of voxels with
4 supra-threshold uptake, reported in ml, and TLG will be calculated as [MTV x SUVmean].

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6 PET2 scans will be evaluated by visual analysis on the basis of Deauville-5-points-scale assigning
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8 Inadequate Response (IR) when at least one site shows FDG uptake higher than liver uptake (scores
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10 4 and 5). Additionally, the variation of SUVmax, determined as the percentage reduction between
11
12 the SUVmax in the tumor site with the most intense uptake on PET1 and the SUVmax in the tumor
13
14 site with the most intense uptake on PET2 (Δ SUVmax) [9], will be computed. Similarly, will be
15
16 calculated the variation of SUVmean, SUVpeak, MTV, and TLG, respectively.

17 18 **Assessment of bulky masses**

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20 The definition of bulky masses will be determined as specified in EuroNet-PHL-C2 [15]. More
21
22 specifically, a volume of a contiguous lymph node mass \geq 200ml, measured by the three largest
23
24 diameters on CT/MRI, will be considered as bulky. All bulky masses will be outlined using
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26 different threshold methods, as explained above, and analyzed on dedicated software for semi-
27
28 quantitative and volumetric parameters. The same software will provide textural and shape features.
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30 SUVmax will be defined as the maximum uptake in the segmented tumor. SUVmean will be
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32 measured as the average uptake in the tumor burden. SUVpeak will be computed as the average
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34 SUV in a 1ml region of tumor burden around the maximal SUV voxel. MTV will be the volume of
35
36 the segmented tumor. TLG will be calculated as the product of SUVmean by MTV.

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38 Among shape parameters, asphericity, convexity and 3D fractal dimensions will be computed [15,
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40 **18, 22, 23**]. For the characterization of tumor texture, two methods will be used as previously
41
42 reported [11, 24, 25]: analysis of the histogram of the voxel values within the tumor and the method
43
44 accounting for the spatial arrangement of voxel values. By histogram-based method, on first-order
45
46 statistics, will be computed SD (standard deviation), Entropy, Energy, kurtosis and Skewness. To
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48 define the spatial arrangement of the voxel values within the tumor, four matrices will be computed
49
50 from each VOI: gray-level co-occurrence matrix (GLCM), neighborhood gray-level different matrix
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52 (NGLDM), gray-level zone length matrix (GLZLM) and gray-level run length matrix (GLRLM).

53 54 **Definition of morphological response**

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56 In pediatric HL patients presenting with morphological partial response on bulky masses and/or
57
58 residual lymph nodes with largest diameter \geq 2 cm, a multiparametric assessment (i.e. SUVmax,
59
60 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: $\geq 30\%$ 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: $\geq 10\%$ 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 (ρ). The different threshold methods used to outline all individual lesions will be compared by the Pearson correlation coefficient, linear regression, Bland-Altman 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

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

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10 In literature there is evidence that metabolically active tumor volume determined by PET/CT is
11 more advantageous than tumor volume measured by CT or MRI for predicting response to
12 treatment in various malignancies afflicting both adult and the pediatric population. More
13 specifically, in adult population, recent publications have demonstrated that the measurement of 3-
14 dimensional disease volume (MTV) and metabolic activity (TLG) [27] can help predict outcomes in
15 HL patients [8, 9, 27-29]. This might suggest a similar implication also in pediatric HL, where the
16 tumor volume may not change because of overlapping inflammatory processes correlated to
17 therapy, while early changes of metabolic activity are most frequently reported.
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20 Along with the above mentioned semi-quantitative parameters, it is possible to extract other
21 quantitative features from PET-CT images, including intensity, heterogeneity, and shape within the
22 tumor, potentially reflecting underlying biological characteristics [27]. These characteristics are
23 embedded in the so called “Radiomics”, a translational field of research aiming to extract high-
24 dimensional data from clinical images to predict underlying biological characteristics of the disease
25 [30]. Radiomic features are correlated to prognostic markers in cancer (i.e. hypoxia, angiogenesis,
26 proliferation, etc.) and might be utilized for tumor response prediction and outcome
27 prognostication. In pediatric population, especially in case of advanced stage disease, high dose
28 therapeutic regimens represent the standard to guarantee cure, yet at the expense of early and
29 delayed side effects [31]. In this context, it becomes even more important to identify those factors
30 capable of limiting the doses to the necessary therapeutic effect while reducing at maximum the
31 undesirable consequences. These prerogatives have guided in the last decades clinical research in
32 adult [32, 33] and pediatric HL [6, 15].
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46 In the present study, we aim to identify prospectively the role of volumetric and texture (radiomic)
47 characteristics better fulfilling the need for predictive and prognostic factors in pediatric HL.
48 Thanks to a large sample size and to a preliminary methodological validation, we expect to obtain
49 significant data on the added value of volumetric and texture analysis on FDG PET assessment in
50 pediatric Hodgkin Lymphoma.
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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.

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LIST OF ABBREVIATIONS

AIEOP = Associazione Italiana di Ematologia e Oncologia Pediatrica

CT = computed tomography

DS = Deauville score

FDG = 18F-fluorodeoxyglucose

GLCM = gray-level co-occurrence 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 PUBLICATION

Not applicable

AVAILABILITY 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.

FUNDING

Fondazione Umberto Veronesi has provided support to the study with three fellowship grants. AGMEN (Associazione Genitori Malati Emato-Oncologici) is supporting the study by covering the expenses for the central revision platform of the trial.

ACKNOWLEDGEMENTS

AIEOP (Associazione Italiana Ematologia Oncologia Pediatrica) Hodgkin Lymphoma Study Group. The authors would like to thank E. Maziotti for the support in the study analyses.

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FIGURE LEGEND

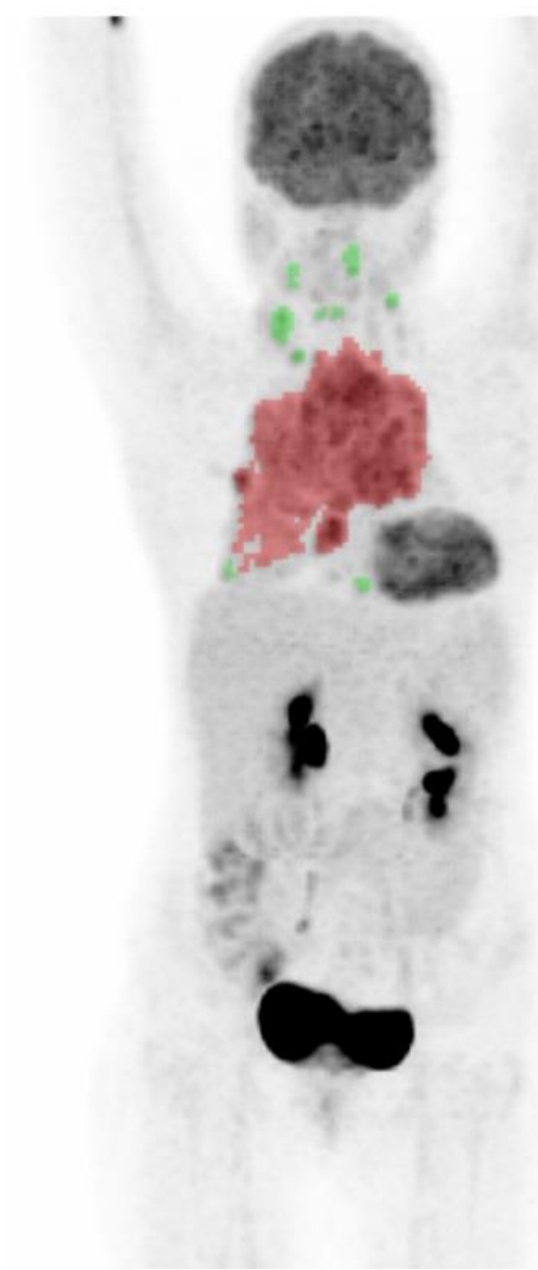
Figure 1: Comparative representation of the four segmentation techniques applied in our study protocol illustrated from left to right: fixed 41% threshold (V41%); fixed absolute SUV threshold of 2.5 (V2.5); SUVmax(lesion)/SUVmean liver >1.5 (Vliver); and adaptative method (AM). The same HL patient has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

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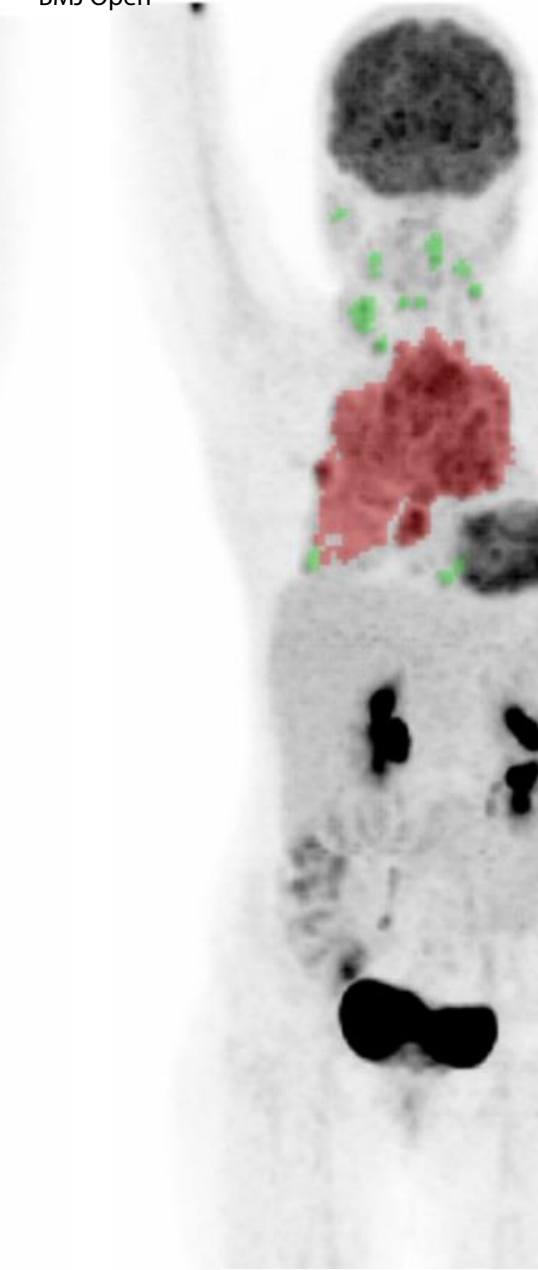
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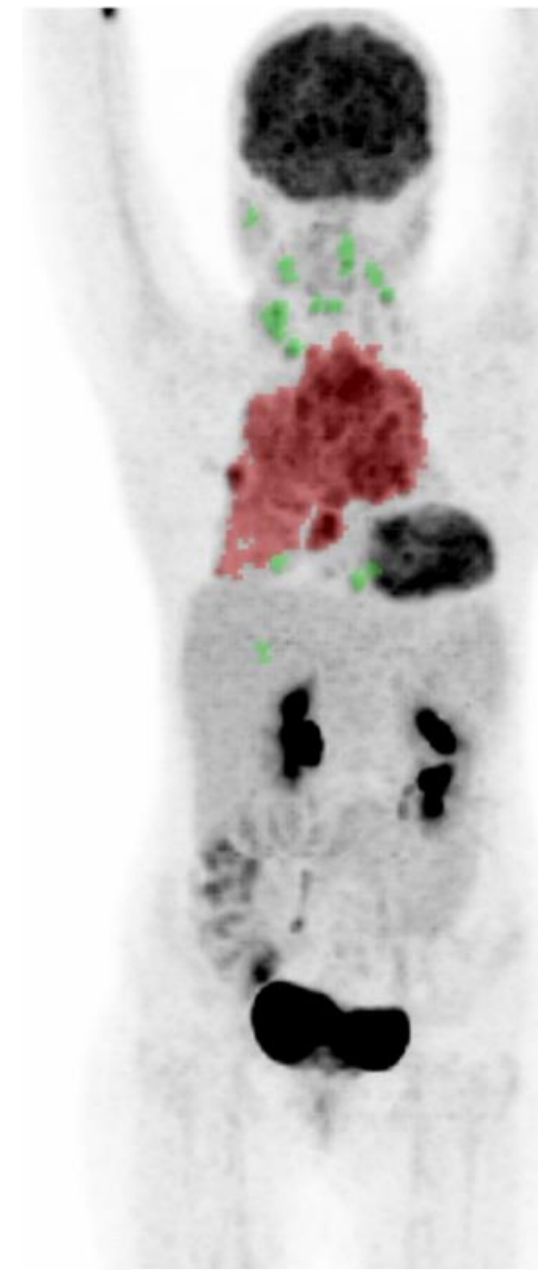
TMTV (V41%) Baseline
242,30



TMTV (V2.5) Baseline
471,00



TMTV (Vliver) Baseline
500,40



TMTV (AM) Baseline
538,3



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

1			
2	Background and	6a	Background, article page 4
3	rationale		
4			
5		6b	Background, article page 4
6			
7	Objectives	7	Study objectives, article page 4
8			
9	Trial design	8	This is a prospective observational multicentric cohort study.
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Methods: Participants, interventions, and outcomes

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16			
17	Study setting	9	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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20	Eligibility criteria	10	Eligibility criteria, article page 5
21			
22	Interventions	11a	N/A
23			
24		11b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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26		11c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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28		11d	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
29			
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33	Outcomes	12	Study objectives, article page
34			
35	Participant	13	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
36	timeline		
37			
38			
39	Sample size	14	Sample size calculation, article page 7
40			
41	Recruitment	15	AIEOP Hodgkin Lymphoma Study Group
42			

Methods: Assignment of interventions (for controlled trials)

Allocation:

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47	Sequence	16a	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
48	generation		
49			
50	Allocation	16b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
51	concealment		
52	mechanism		
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54	Implementation	16c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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58	Blinding	17a	Open label
59	(masking)		
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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

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2	Ancillary and	30	N/A
3	post-trial care		
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5	Dissemination	31a	Trial results will be communicated to participants, healthcare
6	policy		professionals, the public, and other relevant groups via scientific and
7			congress publications.
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9		31b	Authorship eligibility will be provided to investigators based on their
10			contribution to the study.
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12		31c	As per EuroNet-PHL-C2 protocol and AIEOP policy
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Appendices

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16			
17	Informed consent	32	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final
18	materials		version 2017-07-31
19			
20	Biological	33	N/A
21	specimens		
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*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

BMJ Open

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.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-041252.R1
Article Type:	Protocol
Date Submitted by the Author:	18-Nov-2020
Complete List of Authors:	Lopci, Egesta; Humanitas Research Hospital, Nuclear Medicine Department Burnelli, Roberta; University Hospital Arcispedale Sant'Anna of Ferrara, Pediatric Onco-hematologic Unit Elia, Caterina; Centro di Riferimento Oncologico, AYA Oncology and Pediatric Radiotherapy Unit Piccardo, Arnoldo; Ente Ospedaliero Ospedali Galliera, Nuclear Medicine Department Castello, Angelo; Humanitas Research Hospital, Nuclear Medicine Department Borsatti, Eugenio; Centro di Riferimento Oncologico, Nuclear Medicine Department Zucchetta, Pietro; Padua University Hospital, Nuclear Medicine Department Cistaro, Angelina; Ente Ospedaliero Ospedali Galliera, Nuclear Medicine Department Mascarin, Maurizio; Centro di Riferimento Oncologico, AYA Oncology and Pediatric Radiotherapy Unit
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Paediatrics
Keywords:	Lymphoma < HAEMATOLOGY, Nuclear radiology < RADIOLOGY & IMAGING, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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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.

13 Egesta Lopci (1), Roberta Burnelli (2), Caterina Elia (3), Arnoldo Piccardo (4), Angelo
14 Castello (1), Eugenio Borsatti (5), Pietro Zucchetta (6), Angelina Cistaro (4), Maurizio
15 Mascarin (3) and AIEOP Hodgkin Lymphoma Study Group.
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1. Nuclear Medicine department, Humanitas Clinical and Research Hospital – IRCCS, Via Manzoni 56, 20089 Rozzano (MI), Italy;
 2. Pediatric Onco-hematologic Unit, University Hospital S. Anna, Ferrara, Italy.
 3. AYA and Pediatric Radiotherapy, IRCCS Centro di Riferimento Oncologico, Aviano, PN,
 4. Nuclear Medicine, Galliera Hospital, Genoa, Italy.
 5. Nuclear Medicine, Centro di Riferimento Oncologico, Aviano, Pordenone, Italy
 6. Nuclear Medicine Department, University Hospital, Padova, Italy.

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Corresponding author:

Egest Lopci, MD, PhD

Nuclear Medicine Department

Humanitas Clinical and Research Hospital – IRCCS

Via Manzoni 56, CAP 20089, Rozzano (MI), Italy

Email: egesta.lopci@gmail.com

egesta.lopci@cancercenter.humanitas.it

Roberta Burnelli, MD

Pediatric Onco-hematologic Unit

University Hospital S. Anna, Ferrara, Italy

Email: roberta.burnelli@unife.it

Caterina Elia

AYA Oncology and Pediatric Radiotherapy Unit

1
2
3 CRO Centro di Riferimento Oncologico IRCCS, Aviano (PN), Italy

4 Email: eliacaterina@libero.it;
5
6
7

8 Arnaldo Piccardo, MD

9 Nuclear Medicine Department

10 Galliera Hospital, Genoa, Italy.

11 Email: arnaldo.piccardo@galliera.it
12
13
14
15
16

17 Angelo Castello, MD

18 Nuclear Medicine Department

19 Humanitas Clinical and Research Hospital – IRCCS

20 Via Manzoni 56, CAP 20089, Rozzano (MI), Italy

21 Email: angelo.castello@humanitas.it
22
23
24
25
26

27 Eugenio Borsatti, MD

28 Nuclear Medicine Department

29 CRO Centro di Riferimento Oncologico IRCCS, Aviano (PN), Italy

30 Email: eborsatti@cro.it
31
32
33
34
35

36 Pietro Zucchetta, MD

37 Nuclear Medicine Department

38 University Hospital, Padova, Italy

39 Email: pietro.zucchetta@unipd.it
40
41
42
43
44

45 Angelina Cistaro, MD

46 Nuclear Medicine Department

47 Galliera Hospital, Genoa, Italy.

48 Email: angelina.cistaro@galliera.it
49
50
51
52

53 Maurizio Mascarin, MD

54 AYA Oncology and Pediatric Radiotherapy Unit

55 CRO Centro di Riferimento Oncologico IRCCS, Aviano (PN), Italy

56 Email: phl.italy@cro.it; mascarin@cro.it
57
58
59
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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 ≥ 200 ml 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

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

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

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

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

33 The protocol herein illustrated represents a parallel study on PET imaging performed after the
34 EuroNet-PHL-C2 trial amendment (Amendment Nr. 04, dated 2017-07-31) on the Italian cohort of
35 patients. The study has been also submitted and approved by the Italian authority (AIFA) the date
36 2018-03-09. Consequently, the timeline of the protocol will be as follows: I) enrollment period will
37 start from the 10th of March 2018 until 31st of December 2020; II) follow-up period will last 5 years
38 after last enrolment day; III) study completion is planned before 31st December 2025.
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46 **METHODOLOGY**

47 **Whole body assessment of HL**

48 All FDG PET scans performed in the Italian cohort of patients undergoing the EuroNet-PHL-C2
49 trail will be analyzed with additional volumetric assessment comprising metabolic tumor volume
50 (MTV) and total lesion glycolysis (TLG) at baseline and during the course of therapy.
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53 In each patient, HL lesions will be identified by visual analysis and corresponding SUVmax,
54 SUVmean and SUVpeak [18] will be determined as the pixel with the highest value of uptake, the
55 mean value of uptake and the average value of uptake in a VOI (volume of interest) of 1ml that
56 surrounds the voxel with the highest activity, respectively. A dedicated software will be used to
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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;
- $SUV_{max}(\text{lesion})/SUV_{mean \text{ liver}} > 1.5$
- Adaptive method: $I(\text{threshold}) = [0.15 \times I(\text{mean})] + I(\text{background})$. $I(\text{mean})$ is calculated as the mean intensity of all pixels surrounded by the 70% I_{max} isocontour within the tumor; $I(\text{background})$ is defined as a SUV_{mean} 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 $[MTV \times SUV_{mean}]$.

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 (ΔSUV_{max}) [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 $\geq 200\text{ml}$, 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

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3 statistics, will be computed SD (standard deviation), Entropy, Energy, kurtosis and Skewness. To
4 define the spatial arrangement of the voxel values within the tumor, four matrices will be computed
5 from each VOI: gray-level co-occurrence matrix (GLCM), neighborhood gray-level different matrix
6 (NGLDM), gray-level zone length matrix (GLZLM) and gray-level run length matrix (GLRLM).
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8 All parameters obtainable by the software and possible limitations are better detailed at
9 <http://www.lifexsoft.org> [24,25].
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15 **Definition of morphological response**

16 In pediatric HL patients presenting with morphological partial response on bulky masses and/or
17 residual lymph nodes with largest diameter ≥ 2 cm, a multiparametric assessment (i.e. SUVmax,
18 SUVmean, SUVpeak, MTV, TLG and texture analysis) will be performed. For this purpose, the
19 International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017)
20 will be used when necessary [30]. In particular, we will include in the analysis all cases with:
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- 25 • Poor bulk response: $< 50\%$ volume reduction and/or at least one nodal site with largest diameter
26 of ≥ 2 cm and non-assessable qPET-value due to brown fatty tissue [17].
27
- 28 • Partial Response: $\geq 30\%$ decrease in the sum of longest diameters of target lesions but no
29 complete response; positive PET (DS 4–5); any bone marrow involvement, no new lesions [30].
30
- 31 • Minor Response: $\geq 10\%$ decrease in the sum of longest diameters of target lesions but not a
32 partial response [30].
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38 **Sample size calculation**

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

54 No patient involved
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58 **STATISTICAL ANALYSIS**

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

30 31 **DISCUSSION**

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

40
41 Along with the above mentioned semi-quantitative parameters, it is possible to extract other
42 quantitative features from PET-CT images, including intensity, heterogeneity, and shape within the
43 tumor, potentially reflecting underlying biological characteristics [14]. These characteristics are
44 embedded in the so called "Radiomics", a translational field of research aiming to extract high-
45 dimensional data from clinical images to predict underlying biological characteristics of the disease
46 [33]. Radiomic features are correlated to prognostic markers in cancer (i.e. hypoxia, angiogenesis,
47 proliferation, etc.) and might be utilized for tumor response prediction and outcome
48 prognostication. In pediatric population, especially in case of advanced stage disease, high dose
49 therapeutic regimens represent the standard to cure, yet at the expense of early and delayed side
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3 effects [34]. In this context, it becomes even more important to identify those factors capable of
4 limiting the doses to the necessary therapeutic effect while reducing at maximum the undesirable
5 consequences. These prerogatives have guided in the last decades clinical research in adult [35, 36]
6 and pediatric HL [6, 17].
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10 In the present study, we aim to identify prospectively the role of volumetric and texture (radiomic)
11 characteristics better fulfilling the need for predictive and prognostic factors in pediatric HL.
12 Thanks to a large sample size and to a preliminary methodological validation, we expect to obtain
13 significant data on the added value of volumetric and texture analysis on FDG PET assessment in
14 pediatric Hodgkin Lymphoma.
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LIST OF ABBREVIATIONS

AIEOP = Associazione Italiana di Ematologia e Oncologia Pediatrica

CT = computed tomography

DS = Deauville score

FDG = 18F-fluorodeoxyglucose

GLCM = gray-level co-occurrence 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 PUBLICATION

Not applicable

AVAILABILITY 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.

FUNDING

Fondazione Umberto Veronesi has provided support to the study with three fellowship grants. AGMEN (Associazione Genitori Malati Emato-Oncologici) is supporting the study by covering the expenses for the central revision platform of the trial.

ACKNOWLEDGEMENTS

AIEOP (Associazione Italiana Ematologia Oncologia Pediatrica) Hodgkin Lymphoma Study Group. The authors would like to thank E. Maziotti for the support in the study analyses.

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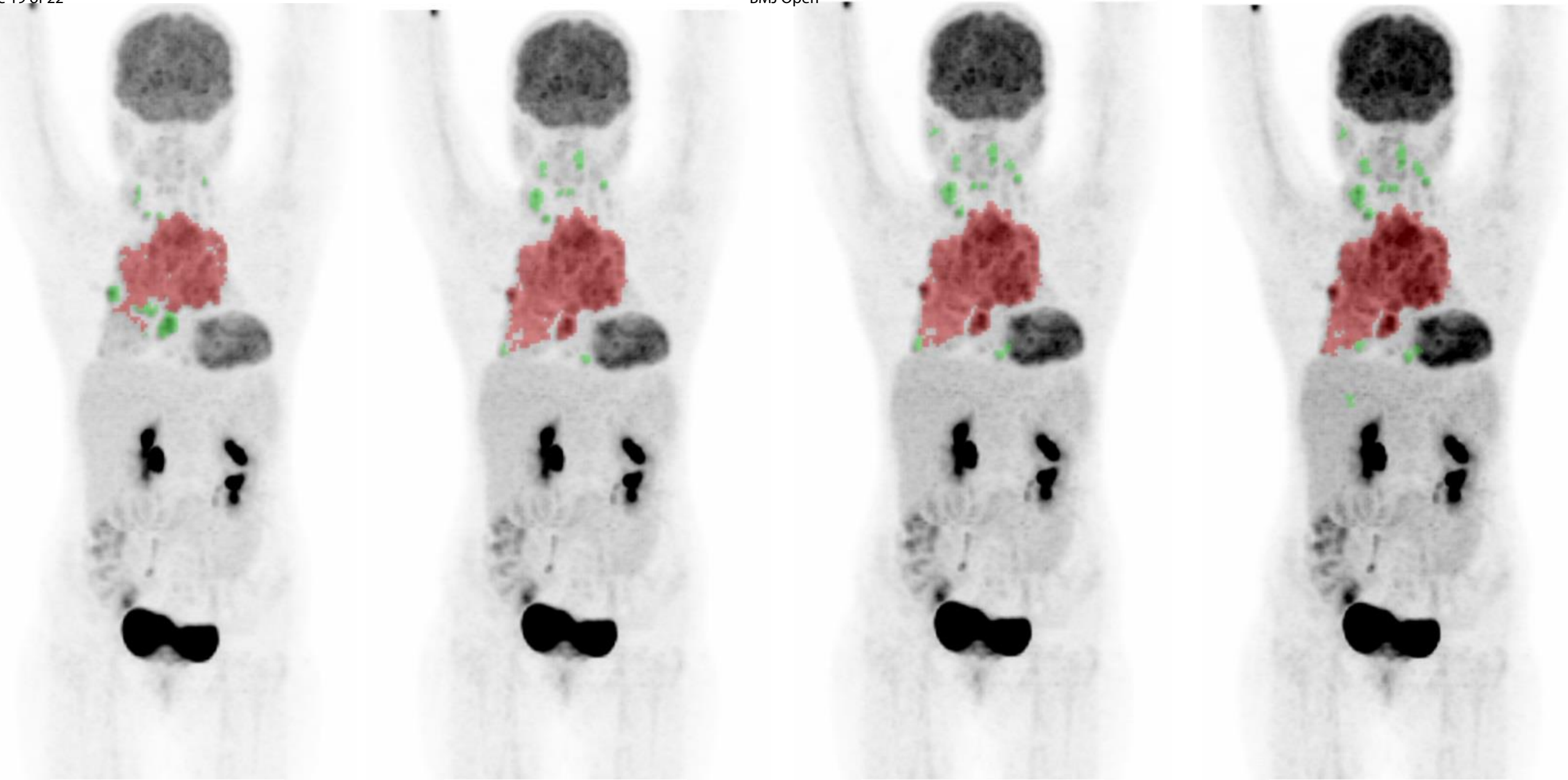
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FIGURE LEGEND

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.

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TMTV (V41%) Baseline
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TMTV (V2.5) Baseline
471,00

TMTV (Vliver) Baseline
500,40

TMTV (AM) Baseline
538,3



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

1			
2	Background and	6a	Background, article page 4
3	rationale		
4			
5		6b	Background, article page 4
6			
7	Objectives	7	Study objectives, article page 4
8			
9	Trial design	8	This is a prospective observational multicentric cohort study.
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Methods: Participants, interventions, and outcomes

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16			
17	Study setting	9	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
18			
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20	Eligibility criteria	10	Eligibility criteria, article page 5
21			
22	Interventions	11a	N/A
23			
24		11b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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26		11c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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28		11d	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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33	Outcomes	12	Study objectives, article page
34			
35	Participant	13	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
36	timeline		
37			
38			
39	Sample size	14	Sample size calculation, article page 7
40			
41	Recruitment	15	AIEOP Hodgkin Lymphoma Study Group
42			

Methods: Assignment of interventions (for controlled trials)

Allocation:

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47	Sequence	16a	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
48	generation		
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50	Allocation	16b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
51	concealment		
52	mechanism		
53			
54	Implementation	16c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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58	Blinding	17a	Open label
59	(masking)		
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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

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2	Ancillary and	30	N/A
3	post-trial care		
4			
5	Dissemination	31a	Trial results will be communicated to participants, healthcare
6	policy		professionals, the public, and other relevant groups via scientific and
7			congress publications.
8			
9		31b	Authorship eligibility will be provided to investigators based on their
10			contribution to the study.
11			
12		31c	As per EuroNet-PHL-C2 protocol and AIEOP policy
13			
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Appendices

15			
16			
17	Informed consent	32	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final
18	materials		version 2017-07-31
19			
20	Biological	33	N/A
21	specimens		
22			

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-041252.R2
Article Type:	Protocol
Date Submitted by the Author:	27-Feb-2021
Complete List of Authors:	Lopci, Egesta; IRCCS Humanitas Research Hospital, Nuclear Medicine Department Burnelli, Roberta; University Hospital Arcispedale Sant'Anna of Ferrara, Pediatric Onco-hematologic Unit Elia, Caterina; Centro di Riferimento Oncologico, AYA Oncology and Pediatric Radiotherapy Unit Piccardo, Arnoldo; Ente Ospedaliero Ospedali Galliera, Nuclear Medicine Department Castello, Angelo; IRCCS Humanitas Research Hospital, Nuclear Medicine Department Borsatti, Eugenio; Centro di Riferimento Oncologico, Nuclear Medicine Department Zucchetta, Pietro; Padua University Hospital, Nuclear Medicine Department Cistaro, Angelina; Ente Ospedaliero Ospedali Galliera, Nuclear Medicine Department Mascarin, Maurizio; Centro di Riferimento Oncologico, AYA Oncology and Pediatric Radiotherapy Unit
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Paediatrics
Keywords:	Lymphoma < HAEMATOLOGY, Nuclear radiology < RADIOLOGY & IMAGING, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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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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Egesta Lopci (1), Roberta Burnelli (2), Caterina Elia (3), Arnoldo Piccardo (4), Angelo Castello (1), Eugenio Borsatti (5), Pietro Zucchetta (6), Angelina Cistaro (4), Maurizio Mascarin (3) and AIEOP Hodgkin Lymphoma Study Group.

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1. Nuclear Medicine department, IRCCS - Humanitas Research Hospital, Via Manzoni 56, 20089 Rozzano (MI), Italy;
 2. Pediatric Onco-hematologic Unit, University Hospital S. Anna, Ferrara, Italy.
 3. AYA and Pediatric Radiotherapy, IRCCS Centro di Riferimento Oncologico, Aviano, PN,
 4. Nuclear Medicine, Galliera Hospital, Genoa, Italy.
 5. Nuclear Medicine, Centro di Riferimento Oncologico, Aviano, Pordenone, Italy
 6. Nuclear Medicine Department, University Hospital, Padova, Italy.

Corresponding author:

Egest Lopci, MD, PhD

Nuclear Medicine Department

IRCCS - Humanitas Research Hospital

Via Manzoni 56, CAP 20089, Rozzano (MI), Italy

Email: egesta.lopci@gmail.com

egesta.lopci@cancercenter.humanitas.it

Roberta Burnelli, MD

Pediatric Onco-hematologic Unit

University Hospital S. Anna, Ferrara, Italy

Email: roberta.burnelli@unife.it

Caterina Elia

AYA Oncology and Pediatric Radiotherapy Unit

CRO Centro di Riferimento Oncologico IRCCS, Aviano (PN), Italy

Email: eliacaterina@libero.it

1
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4
5 Arnoldo Piccardo, MD
6 Nuclear Medicine Department
7 Galliera Hospital, Genoa, Italy.
8
9 Email: arnoldo.piccardo@galliera.it
10
11
12

13 Angelo Castello, MD
14 Nuclear Medicine Department
15 IRCCS - Humanitas Research Hospital
16
17 Via Manzoni 56, CAP 20089, Rozzano (MI), Italy
18
19 Email: angelo.castello@humanitas.it
20
21
22

23 Eugenio Borsatti, MD
24 Nuclear Medicine Department
25
26 CRO Centro di Riferimento Oncologico IRCCS, Aviano (PN), Italy
27
28 Email: eborsatti@cro.it
29
30
31

32 Pietro Zucchetta, MD
33 Nuclear Medicine Department
34
35 University Hospital, Padova, Italy
36
37 Email: pietro.zucchetta@unipd.it
38
39
40

41 Angelina Cistaro, MD
42 Nuclear Medicine Department
43
44 Galliera Hospital, Genoa, Italy.
45
46 Email: angelina.cistaro@galliera.it
47
48
49

50 Maurizio Mascarin, MD
51 AYA Oncology and Pediatric Radiotherapy Unit
52
53 CRO Centro di Riferimento Oncologico IRCCS, Aviano (PN), Italy
54
55 Email: phl.italy@cro.it; mascarin@cro.it
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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 ≥ 200 ml 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

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⁵ ¹⁸F-fluorodeoxyglucose positron emission tomography (FDG PET) has become a standard
⁶ diagnostic procedure for the assessment of response to therapy in adults and children with Hodgkin
⁷ lymphoma. International guidelines recommend the using of Deauville five-point scale as a visual
⁸ method for discriminating responders from non-responder patients [1, 2]. In 2014, the pediatric
⁹ German group proposed the use of qPET with the intent to extend the Deauville score to a
¹⁰ continuous scale and limit optical misinterpretation due to the influence of background activity [3,
¹¹ 4]. This quantitative method is being applied in the current EuroNet-PHL-C2 clinical trial, in which
¹² adapted therapy is based on quantitative FDG avidity of tumor masses on PET evaluation after 2
¹³ cycles of OEPA [5, 6]. This approach, however, postpones risk stratification at interim evaluation;
¹⁴ therefore, the definition of imaging baseline predictors is highly desirable.

¹⁵ The implementation of metabolic tumor volume, as a sum of areas with an increased SUV inside
¹⁶ the tumor, as well as the characterization of the heterogeneity of tumor metabolic patterns on FDG
¹⁷ PET has become an emerging topic in nuclear medicine [7]. Several studies [8-11] have shown that
¹⁸ the addition of volumetric and textural parameters can be a valuable help for disease
¹⁹ prognostication and biological characterization of many tumor types, thus suggesting a similar
²⁰ implication for pediatric Hodgkin lymphoma [12]. While the concept of “Radiomics”, consisting on
²¹ the extraction of a large quantity of features from digital images via data-characterization
²² algorithms has gained a proper place in predicting outcome and early metabolic response in adults
²³ with malignant lymphoma [13, 14] On the other hand, the scientific background and the results
²⁴ obtained from our previous studies in the context of the Italian AIEOP-LH2004 trial [15, 16]
²⁵ suggest an additional impact of FDG PET in patients with or without bulky disease presenting with
²⁶ residual masses on morphological evaluation with computed tomography (CT).

²⁷ Given the abovementioned premises, the AIEOP Hodgkin Lymphoma Study Group has planned to
²⁸ perform in the Italian cohort of patients treated according to the EuroNet-PHL-C2 trial additional
²⁹ volumetric analyses to improve the evaluation of tumor burden computed at baseline FDG PET and
³⁰ to identify prognostic factors suitable for predicting early metabolic response to therapy in pediatric
³¹ Hodgkin lymphoma (HL). In case of bulky disease, further textural and shape analysis in the
³² baseline FDG PET will be performed to evaluate macroscopic and microscopic heterogeneity of
³³ tumor masses, as reflection of their aggressiveness and different chemotherapy sensitivity.
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METHODS AND ANALYSIS

STUDY OBJECTIVES

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3 This is a prospective observational multicentric cohort study. The primary objective of the study is
4 to compare the diagnostic and prognostic role of volumetric assessment in HL patients at baseline
5 and during the course of therapy with standard visual (Deauville score) and semi-quantitative (i.e.
6 SUVmax, SUVmean, SUVpeak) analyses.
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10 The secondary objective of the study is to identify the diagnostic and prognostic impact of texture
11 analyses and the other metabolic parameters on bulky masses.
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13 The tertiary objective of the study is to determine the additional predictive and prognostic value of
14 multiparametric assessment (i.e. SUVmax, SUVmean, SUVpeak, MTV, TLG and texture analysis)
15 in HL patients having a partial response on morphological imaging.
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19 20 ELIGIBILITY CRITERIA

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

37 The protocol herein illustrated represents a parallel study on PET imaging performed after the
38 EuroNet-PHL-C2 trial amendment (Amendment Nr. 04, dated 2017-07-31) on the Italian cohort of
39 patients. The study has been also submitted and approved by the Italian authority (AIFA) the date
40 2018-03-09. Consequently, the timeline of the protocol will be as follows: I) enrollment period will
41 start from the 10th of March 2018 until 31st of December 2020; II) follow-up period will last 5 years
42 after last enrolment day; III) study completion is planned before 31st December 2025.
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50 METHODOLOGY

51 **Whole body assessment of HL**

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53 All FDG PET scans performed in the Italian cohort of patients undergoing the EuroNet-PHL-C2
54 trail will be analyzed with additional volumetric assessment comprising metabolic tumor volume
55 (MTV) and total lesion glycolysis (TLG) at baseline and during the course of therapy.
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58 In each patient, HL lesions will be identified by visual analysis and corresponding SUVmax,
59 SUVmean and SUVpeak [18] will be determined as the pixel with the highest value of uptake, the
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3 mean value of uptake and the average value of uptake in a VOI (volume of interest) of 1ml that
4 surrounds the voxel with the highest activity, respectively. A dedicated software will be used to
5 delineate, semi-automatically, contours of the lesions using different threshold methods and the
6 impact of segmentation technique will be evaluated (**Figure 1**). More specifically, four threshold
7 methods will be used based on previously reported methodologies [19-22]:
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- 10 • Fixed 41% threshold of the SUVmax within the respective lymphoma site,
- 11 • Fixed absolute SUV threshold of 2.5;
- 12 • SUVmax(lesion)/SUVmean liver >1.5
- 13 • Adaptive method: $I(\text{threshold}) = [0.15 \times I(\text{mean})] + I(\text{background})$. $I(\text{mean})$ is calculated as the
14 mean intensity of all pixels surrounded by the 70% I_{max} isocontour within the tumor;
15 $I(\text{background})$ is defined as a SUVmean of liver [23].

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

19 PET2 scans will be evaluated by visual analysis on the basis of Deauville-5-points-scale assigning
20 Inadequate Response (IR) when at least one site shows FDG uptake higher than liver uptake (scores
21 4 and 5). Additionally, the variation of SUVmax, determined as the percentage reduction between
22 the SUVmax in the tumor site with the most intense uptake on PET1 and the SUVmax in the tumor
23 site with the most intense uptake on PET2 (ΔSUVmax) [9], will be computed. Similarly, will be
24 calculated the variation of SUVmean, SUVpeak, MTV, and TLG, respectively.

25 26 27 28 29 30 31 32 33 34 35 36 37 38 **Assessment of bulky masses and radiomics analyses**

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

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

50 Among shape parameters, asphericity, convexity and 3D fractal dimensions will be computed [15,
51 18, 26, 27]. For the characterization of tumor texture, two methods will be used as previously

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

20 In pediatric HL patients presenting with morphological partial response on bulky masses and/or
21 residual lymph nodes with largest diameter ≥ 2 cm, a multiparametric assessment (i.e. SUVmax,
22 SUVmean, SUVpeak, MTV, TLG and texture analysis) will be performed. For this purpose, the
23 International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017)
24 will be used when necessary [30]. In particular, we will include in the analysis all cases with:
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- 29 • Poor bulk response: $< 50\%$ volume reduction and/or at least one nodal site with largest diameter
30 of ≥ 2 cm and non-assessable qPET-value due to brown fatty tissue [17].
- 31 • Partial Response: $\geq 30\%$ decrease in the sum of longest diameters of target lesions but no
32 complete response; positive PET (DS 4–5); any bone marrow involvement, no new lesions [30].
- 33 • Minor Response: $\geq 10\%$ decrease in the sum of longest diameters of target lesions but not a
34 partial response [30].
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41 **Sample size calculation**

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

58 No patient involved
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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 (ρ). The different threshold methods used to outline all individual lesions will be compared by the Pearson correlation coefficient, linear regression, Bland-Altman 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

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3 therapeutic regimens represent the standard to cure, yet at the expense of early and delayed side
4 effects [34]. In this context, it becomes even more important to identify those factors capable of
5 limiting the doses to the necessary therapeutic effect while reducing at maximum the undesirable
6 consequences. These prerogatives have guided in the last decades clinical research in adult [35, 36]
7 and pediatric HL [6, 17].
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10 In the present study, we aim to identify prospectively the role of volumetric and texture (radiomic)
11 characteristics better fulfilling the need for predictive and prognostic factors in pediatric HL.
12 Thanks to a large sample size and to a preliminary methodological validation, we expect to obtain
13 significant data on the added value of volumetric and texture analysis on FDG PET assessment in
14 pediatric Hodgkin Lymphoma.
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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 will be actively disseminated through peer-reviewed journals, conference presentations, social media, print media and internet.

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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; Arnaldo Piccardo, Angelo Castello, Eugenio Borsatti, Pietro Zucchetto 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.

FUNDING

Fondazione Umberto Veronesi has provided support to the study with three fellowship grants. AGMEN (Associazione Genitori Malati Emato-Oncologici) is supporting the study by covering the expenses for the central revision platform of the trial.

ACKNOWLEDGEMENTS

AIEOP (Associazione Italiana Ematologia Oncologia Pediatrica) Hodgkin Lymphoma Study Group. The authors would like to thank E. Mazziotti for the support in the study analyses.

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.

For peer review only

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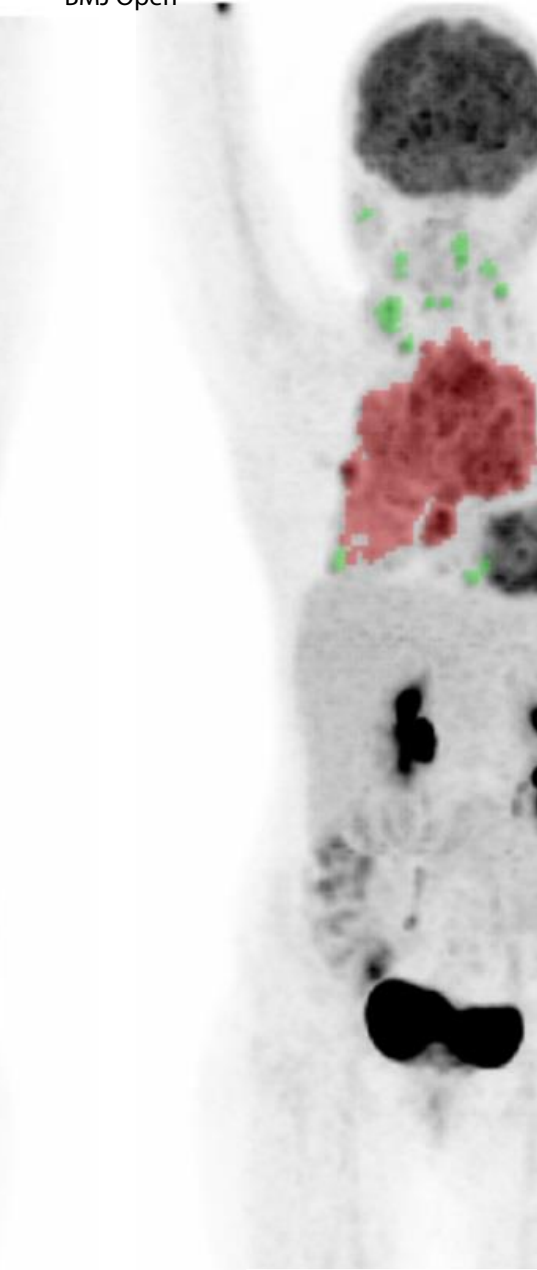
TMTV (V41%) Baseline

242,30



TMTV (V2.5) Baseline

471,00



TMTV (Vliver) Baseline

500,40



TMTV (AM) Baseline

538,3



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

1			
2	Background and	6a	Background, article page 4
3	rationale		
4			
5		6b	Background, article page 4
6			
7	Objectives	7	Study objectives, article page 4
8			
9	Trial design	8	This is a prospective observational multicentric cohort study.
10			
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Methods: Participants, interventions, and outcomes

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16			
17	Study setting	9	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
18			
19			
20	Eligibility criteria	10	Eligibility criteria, article page 5
21			
22	Interventions	11a	N/A
23			
24		11b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
25			
26		11c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
27			
28		11d	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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33	Outcomes	12	Study objectives, article page
34			
35	Participant	13	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
36	timeline		
37			
38			
39	Sample size	14	Sample size calculation, article page 7
40			
41	Recruitment	15	AIEOP Hodgkin Lymphoma Study Group
42			

Methods: Assignment of interventions (for controlled trials)

Allocation:

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47	Sequence	16a	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
48	generation		
49			
50	Allocation	16b	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
51	concealment		
52	mechanism		
53			
54	Implementation	16c	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final version 2017-07-31
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58	Blinding	17a	Open label
59	(masking)		
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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

1			
2	Ancillary and	30	N/A
3	post-trial care		
4			
5	Dissemination	31a	Trial results will be communicated to participants, healthcare
6	policy		professionals, the public, and other relevant groups via scientific and
7			congress publications.
8			
9		31b	Authorship eligibility will be provided to investigators based on their
10			contribution to the study.
11			
12		31c	As per EuroNet-PHL-C2 protocol and AIEOP policy
13			
14			

Appendices

15			
16			
17	Informed consent	32	As per EuroNet-PHL-C2 amendment Nr. 04 to the protocol 4.0 final
18	materials		version 2017-07-31
19			
20	Biological	33	N/A
21	specimens		
22			

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.