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Original Research

2-deoxy-2[F-18] fluoro-D-GLUCOSE POSITRON EMISSION TOMOGRAPHY Deauville scale and core-needle biopsy to determine successful management after six doxorubicin, bleomycin, vinblastine and dacarbazine cycles in advanced-stage Hodgkin lymphoma

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Deauville scale score;
Core-needle biopsy

Abstract ***Background:*** The clinical impact of the positivity of the Deauville scale (DS) of positron emission tomography (PET) performed at the end of doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) in patients with advanced Hodgkin lymphoma (HL), in terms of providing rationale to shift poor responders onto a more intensive regimen, remain to be validated by histopathology.

Patients and methods: This prospective trial involved patients with stage IIB/IV HL who after six ABVD cycles underwent PET (PET6) and core-needle cutting biopsy (CNCB) of 2-deoxy-2 [F-18] fluoro-D-glucose (FDG)-avid lymph nodes. Patients received high-dose chemotherapy/ autologous haematopoietic stem cell rescue (HDCT/AHSCR) if CNCB was positive for HL, alternatively, if CNCB or PET was negative, received observation or consolidation radiotherapy (cRT) on residual nodal masses, as initially planned. The end-point was 5-year progression-free survival (PFS).

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Results: In all, 43 of the 169 (25%) evaluable patients were PET6 positive (DS 4, 32; DS 5, 11). Among them, histology showed malignancy (HL) in 100% of DS 5 scores and in 12.5% of DS 4 scores. Fifteen patients with positive biopsy received HDCT/AHSCR, whereas 28 patients with negative biopsy, as well as 126 patients with negative PET6, continued the original plan (cRT, 78 patients; observation, 76 patients). The 5-year PFS in the negative PET6 group, negative biopsy group and positive-biopsy group was 95.4%, 100% and 52.5%, respectively.
Conclusion: DS positivity of end-of-ABVD PET in advanced HL carried a certain number of CNCB-proven non-malignant FDG-uptakes. The DS 4 scores which were found to have negative histology appeared to benefit from continuing the original non-intensive therapeutic plane as indicated by the successful outcome in more than 95% of them by obtaining similar 5-year PFS to the PET6-negative group. By contrast, the DS 5 score had consistently positive histology and was associated with unsuccessful conventional therapy, promptly requiring treatment intensification or innovative therapeutic approaches.

1. Introduction

2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography (FDG-PET) is considered the standard of care for remission assessment in Hodgkin lymphoma (HL) at unfavourable prognosis [1]. At the end of the frontline doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) program, it is crucial to distinguish the responders from non-responders [2]. These latter require treatment intensification including salvage regimens and haematopoietic stem cell (HSC) transplantation [3]. Such intensive approach has shown superior disease control with 5-year freedom from treatment failure achieved in up to 50% of patients [2,3] but at the cost of an increased haematologic toxicity, a high incidence of sterility and long-term complications [4]. Exact data on the diagnostic accuracy of end-of-treatment positive positron emission tomography (PET) are missing [5]. Many studies on this issue are methodologically flawed because they confirm disease persistence by means of imaging monitoring [6]. A review and meta-analysis performed by Adams and Kwee [7] found only four original studies (2 with prospective data acquisition, and 2 with retrospective data acquisition) which routinely performed biopsies of post-chemotherapy 2-deoxy-2[F-18] fluoro-D-glucose (FDG)–avid residual lesions (two studies with standardised criteria and two studies with non-standardised criteria, for defining PET positivity) in a total of 42 patients with HL [8–11]. By analysing the data, the authors showed that the pooled summary false-positive rate of end-of-treatment FDG-PET in such instances was 35% with a range of 8%–65% [7–11].

To avoid overtreatment, attempts to improve PET-oriented therapy are welcome [1,12,13]. The introduction of the new generation of ultrasonography (US)/computed tomography (CT) and biopsy needle devices provides the opportunity to develop miniinvasive and effective combined diagnostic strategy, avoiding psychological and physical pain of surgical intervention, and increasing consent by patients (and treating physicians) to histologically characterise lesions suspected at FDG-PET [14].

This study, launched in 2009, was a response-adapted strategy that incorporated both FDG-PET and core-needle cutting biopsy (CNCB) to accurately manage patients with advanced HL after the completion of conventional front-line chemotherapy [2].

2. Patients and Methods

2.1. Study design and participants

This prospective trial was designed to determine whether at the end of chemotherapy, decision-making based on the combination of the Deauville scale (DS) 5-point scoring system for interpreting FDG-PET scans [12,13] and core-needle biopsy [14] could better identify patients promptly requiring high-dose chemotherapy/autologous HSC rescue (HDCT/AHSCR) as part of the treatment of advanced HL [2,3] and to determine whether HDCT/AHSCR could be safely avoided in patients with a negative core-needle biopsy of FDG-avid residual lesions, as well as in patients with negative FDG-PET.

Patients had to fulfil the following inclusion criteria: histopathologic diagnosis of classical HL [15], aged 18–60 years, no previous antilymphomatous treatment, Ann Arbor stage IIB to IV [2], conventional induction therapy planned with intent-to-cure (including combined modality treatment: as chemotherapy program six full cycles of ABVD, optionally followed by localised irradiation) [2,16,17] and restaging with FDG-PET interpreted according to the 5-point DS score [13].

The study was conducted in the Haematology Unit of the Federico II University of Naples (Italy). All necessary approvals were obtained from our ethics committee, and a specific consent form dedicated to

interventional procedures was obtained from each patient according to the Declaration of Helsinki.

2.2. PET imaging

FDG-PET examinations were conducted as previously described [16,18]. All patients underwent, on a single occasion, whole-body FDG-PET/CT with a combined in-line system (Discovery LS; GE Medical Systems, Milwaukee, WI) that integrates a four-detector row spiral CT (Lightspeed Ultra; GE Medical Systems) with a PET scanner (Discovery LS8; GE Medical Systems). FDG was produced in on-site cyclotron and chemistry facilities. A dose of 5.3 ± 1 MBq/kg of FDG was intravenous (i.v.) injected 60 ± 10 min before imaging. PET scans were carried out from the midbrain to the upper thigh after an 8-h fast, with 2D emission scans of 4 min per bed position. Images (128 x 128 matrix) were obtained with an iterative ordered subset expectation maximisation (OSEM). The OSEM algorithm was applied to ratio sinograms using attenuation-weighted iterative reconstruction (two iterations, 28 subsets) and subsequent smoothing with a Hanning filter. Comparable axial resolutions (full width at half maximum 5.45 mm) were obtained according to the National Electrical Manufacturers Association NU 2001 test procedure. Unenhanced low-dose CT for segmented attenuation correction was carried out in each patient; immediately after, PET scans were acquired covering the same field of view as the CT. FDG-PET and CT data sets were viewed in fused mode using a special fusion workstation.

A panel of four nuclear medicine experts (R. Fonti, E. Nicolai, C. Mainolfi and S. Del Vecchio) reviewed all restaging scans alongside baseline scans to verify abnormal uptakes in residual or new lymph nodes. FDG-PET results were reported according to the DS score using visual assessment [12,13] followed by quantitative verification as already described [1]. In particular, DS scores were assigned as follows: score 1, no residual uptake; score 2, uptake ≤ mediastinum; score 3, uptake > mediastinum but ≤ liver; score 4, uptake moderately > liver [up to twice the maximal standardised uptake value (SUV_{max}) in a large region of normal liver]; score 5, uptake markedly increased than liver (more than twice the SUV_{max} in a large region of normal liver) and/or new lesions. A negative restaging PET scan was defined as DS ≤ 3 score, and a positive restaging PET scan was defined as DS 4–5 score.

2.3. Biopsy procedures

Those patients who presented positive PET at the end of ABVD PET were targeted to receive histopathology verification. The main criterion for selecting the node to be biopsied was the highest SUV_{max} at FDG-PET scans [1,12,13]. Under US or CT guidance, CNCB was carried out with aseptic technique and cutaneous anaesthesia, using a 16-gauge diameter modified Menghini needle 150 mm in length with automatic aspiration (Biomol® HS-Hospital; Rome, Italy) in accordance with methods already described [14,19,20]. CNCBs were performed by M. Picardi and R. Della Pepa (with more than 10 years of experience with interventional US procedures) and P. Venetucci (with more than 10 years of experience with interventional CT procedures).

Histopathologic examination of core-needle lymph node samples was performed in a single pathology unit by three expert hematopathologists (M. Mascolo, E. Vigliar and/or G. Troncone, with more than 10 years of experience with hematopathological analysis).

2.4. Management plan after ABVD

After restaging diagnostic work-up including FDG-PET and CNCB if needed [2,16,19,20], for the study purpose, only patients with histologically confirmed residual disease promptly underwent ifosfamide-based salvage chemotherapy for 2–4 cycles followed by autologous HSC mobilisation and transplantation (conditioning regimen: carmustine, etoposide, cytarabine and melphalan or fotemustine, etoposide, cytarabine and melphalan) [2,3]. Whereas, the remaining patients (those with negative PET and those with positive PET/negative biopsy) did not modify the initially planned therapeutic strategy. In particular, they received either observation or (for those cases with initial large nodal mass [defined as systemic adenopathy with the largest diameter≥ 5 cm]) [17] consolidation radiotherapy (cRT) at 30 Gy on residual bulky area, that is, containing nodes of size≥ 2.0 cm at CT scans [2,16]. During follow-up, FDG-PET scans were performed every 3–6 months.

2.5. Statistical analysis

This prospective trial aimed to provide an optimal benefit in terms of 5-year progression-free survival (PFS) of a PET plus CNCB response–adapted strategy following the ABVD program in advanced HL [2]. The end-point of the study was PFS, defined as the interval from the date of registration until the date of the first appearance (after the end-of-ABVD FDG-PET) of disease progression/unresponsiveness, relapse or death from any cause or the last follow-up. A minimum of 154 patients was needed for enrolment according to the Simon's optimal two-stage design, with an α-error of 5%, a power of 80% and an expectation to cure of approximately 90% at 5-year follow-up [2,3]. The probabilities of being progression free after therapy were calculated based on Kaplan-Meier estimates (log-rank test). The overall survival (OS) was also reported; it was measured from the date of registration to the date of death as result of any cause.

Patients' characteristics were compared according to restaging PET and CNCB results by using the Mann-

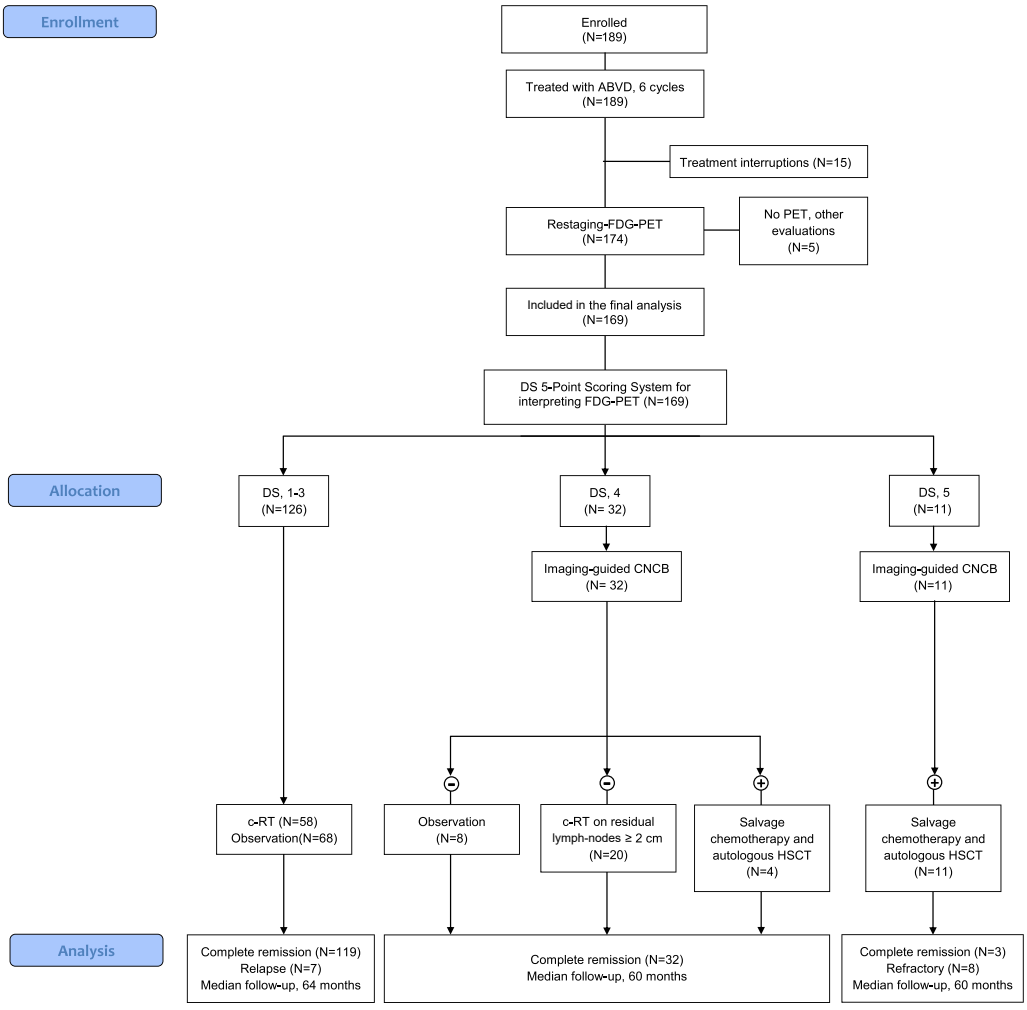

Fig. 1. Consort diagram. After ABVD program, according to the combined diagnostic work-up with FDG-PET and CNCB, 9% patients underwent autologous HSCT, 46% patients underwent c-RT and 45% patients underwent observation. ABVD = doxorubicin, bleomycin, vinblastine and dacarbazine; FDG-PET = 2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography; DS = Deauville scale scores = 1, no uptake above background; 2, uptake ≤ mediastinum; 3, uptake > mediastinum but ≤ liver; 4, uptake moderately > liver; 5, uptake markedly higher than liver and/or new lesions (references #1, 12, 13); CNCB = core-needle cutting biopsy; c-RT = consolidation radiotherapy; HSCT = haematopoietic stem cell transplantation.

Whitney U test and χ^2 test (or Fisher's exact test, if appropriate) for continuous variables and categorical variables, respectively. For safety analyses, frequency of toxicity was reported by the type and grade according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.03.

3. Results

3.1. Recruitment and patient characteristics

In all, 189 patients who were registered into the study started ABVD treatment from September 2009 to December 2017. Of those patients, 169 underwent six ABVD cycles and restaging FDG-PET as scheduled, thus constituting the entire population included in the final assessment. The remaining twenty patients (10.5%) were excluded for several reasons: fifteen patients interrupted the treatment before the end of six ABVD cycles (7 cases withdrew consent; 8 cases were lost to follow-up) and erroneously five patients did not receive restaging with FDG-PET. The entire study flow is shown in Fig. 1. The median age was 31 years (range, 18–60); 53% were women; 38% had stage IIB, 32% stage III and 30% stage IV HL. B symptoms were recorded in 61% and large nodal masses in 60% of patients (Table 1).

3.2. FDG-PET at restaging post-ABVD

The four reviewers unanimously agreed on 90% of restaging PET scans, whereas with the remaining 10% scans, most reviewers (i.e. three of four) defined the final assessment. Among the readers, there was perfect consensus on all scans defined as DS 5 scores,

Table 1
Characteristics of patients included in the study, according to the combined FDG-PET and CNCB results at restaging.

Characteristics	All patients	Negative PET	Positive PET/negative biopsy	Positive PET/positive biopsy
No. of patients	169	126	28	15
median age (range), years	31 (18–60)	32 (18–56)	27 (15–52)	27 (16–60)
Sex				
Male	80 (47)	63 (50)	13 (46)	4 (27)
Female	89 (53)	63 (50)	15 (54)	11 (73)
Hodgkin lymphoma subtypes				
Nodular sclerosis	130 (77)	99 (78)	20 (71)	11 (73)
Mixed cellularity	32 (19)	22 (17)	6 (21)	4 (27)
Lymphocyte rich	5 (3)	4 (3)	1 (4)	–
Lymphocyte depleted	2 (1)	1 (2)	1 (4)	–
Ann Arbor Stage				
II-B	65 (38)	50 (40)	9 (32)	6 (40)
III-VI	104 (62)	76 (60)	19 (68)	9 (60)
IPS				
0–1	60 (36)	50 (40)	10 (35)	–
2–3	86 (51)	63 (50)	13 (45)	10 (66)
> 3	23 (13)	13 (10)	5 (20)	5 (34)
Bulky disease	102 (60)	73 (58)	20 (71)	9 (60)

Values are n (%) unless otherwise noted.
Ann Arbor Stage II is defined as involvement of 2 or more nodal groups on the same side of the diaphragm; stage III is defined as involvement of nodal groups on both sides of the diaphragm and stage IV is defined as disseminated involvement of 1 or more extralymphatic organs (e.g. lung, bone) with or without any nodal involvement.
Bulky disease is defined as any mass with size ≥5 cm on computed tomography or ultrasonography scans (reference #17).
B symptoms are defined as systemic symptoms of fever, night sweats and weight loss which can be associated with Hodgkin lymphoma.
FDG-PET = 2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography; CNCB = core-needle cutting biopsy; IPS = International Prognostic Score (reference #2).

disagreement occurred for 17 scans which were finally defined as DS 4 (n = 10) and 3 (n = 7) scores.

Restaging PET assessments were performed at a median of 4 weeks (range, 4–6) after completion of induction chemotherapy with ABVD program. Thereafter, 126 (75%) patients were found to have a negative end-of-treatment PET and 43 (25%) patients were found to have a positive end-of-treatment PET. The analysis of the FDG-imaging scans of 169 patients assigned a DS as follows: score 1 to 99 patients, score 2 to 3 patients, score 3 to 24 patients, score 4 to 32 patients and score 5 to 11 patients (Fig. 1). At coregistration CT assessments, the median of residual lymph node long axis diameters was 2.1 cm (range, 1.0–2.5 cm) for negative PETs and 3 cm (range, 1.8–4.2 cm) for positive PETs.

Among the 43 patients with positive PET (DS ≥ 4 scores), FDG focal uptakes were localised in two or more non-contiguous lymph nodal sites in 58% (n = 25) of patients (neck on both sides, 8 cases; neck + armpit cable, 3 cases; neck + mediastinum, 10 cases; para-aortic + iliac, 3 cases; inguinal on both sides, 1 case), in new extranodal sites plus lymph nodal areas (lung + iliac, 1 case; liver + para-aortic, 1 case; bone + inguinal, 1 case) in 7% (n = 3) of patients and in one lymph nodal site in 35% (n = 15) of patients (neck, 6 cases; mediastinum, 8 cases; inguinal, 1 case).

3.3. Imaging-guided CNCB

After written informed consent, 43 patients with positive PET at restaging received imaging-guided biopsy. In all patients, FDG-avid residual tissue which was biopsied had ≥1.8 cm long axis and was seated at <15 cm from the skin.

Table 2 shows details regarding CNCB sites. In particular, 25 (58%) patients underwent biopsy in the neck compartments, 10 (23%) patients underwent biopsy in the mediastinum compartments and 8 (19%) patients underwent biopsy in the abdominal-pelvic compartments. All patients underwent CNCB in a day hospital regimen under local anaesthesia except 10 patients (the ones biopsied in the mediastinum compartments) who received procedures with an average hospitalisation of 2 days. No patients suffered from biopsy procedure–related complications of grade ≥2 according to the CTCAE.

Table 2 shows details regarding CNCB pathological findings. Adequate core-needle specimens were obtained in all cases. At histopathology, 15 (35%) patients were found to have lymph nodes positive for HL persistence and 28 (65%) patients to have lymph nodes negative for malignancy. Of the 28 cases with biopsy-proven lymph nodes negative for malignancy, sarcoid-like granulomatosis associated with steatofibrotic and/or necrotic changes was the most common histological feature occurring in 53.5% of patients (15/28 cases). The remaining histologic findings were florid reactive follicular hyperplasia occurring in 39% (11/28 cases) and progressive transformation of germinal centre occurring in 7.5% (2/28 cases).

Among the 15 patients with positive PET/positive biopsy, only one (7%) patient had a single lymph node

Table 2
Core-needle cutting biopsy and FDG-PET findings at restaging.

Characteristics	All patients	Positive-PET/negative biopsy	Positive PET/positive biopsy	p-value
No. of patients	43	28	15	–
Deauville scale scores				
4	32 (74)	28 (100)	4 (27)	–
5	11 (26)	–	11 (73)	–
Residual lymph node long axis, cm				
Median (range)	3 (1.8–4.2)	2.5 (1.8–3.2)	3.5 (2.0–4.2)	0.06
Highest SUV at biopsy site				
Median (range)	6.5 (4–19.6)	4.5 (4–9)	9 (5.4–19.6)	0.003
Site of biopsed lymph node				
Cervical	10 (23)	8 (29)	2 (13)	0.451
Clavicular	15 (35)	9 (32)	6 (40)	0.739
Mediastinum	10 (23)	5 (18)	5 (33)	0.281
Abdominal	3 (7)	2 (7)	1 (7)	1.0
Pelvis	5 (12)	4 (14)	1 (7)	0.636
Imaging guidance				
Ultrasonography	33 (77)	23 (82)	10 (67)	0.281
Computed tomography	10 (23)	5 (18)	5 (33)	
Average time for CNCB, minutes				
Median (range)	40 (30–50)	35 (30–50)	40 (30–50)	0.584
Core-needle passes				
Median (range)	2 (1–4)	2 (1–4)	2 (1–4)	0.967
Core-needle specimen				
Median length (range), mm	30 (15–40)	25 (15–30)	33 (15–40)	0.524
Median estimated volume (range), mm^3	185 (92–430)	180 (92–330)	190 (92–430)	0.349
Histological findings				
Classic Hodgkin lymphoma	15 (35)	–	15 (100)	–
Sarcoid-like granulomatosis	15 (30)	15 (53.5)	–	–
Florid reactive follicular hyperplasia	11 (28)	11 (39)	–	–
Progressive transformation of germinal centre	2 (7)	2 (7.5)	–	0.535
Most common CNCB-related adverse events				
Pain on biopsy site	11 (6)	4 (14)	7 (47)	0.064
Haematoma	3 (2)	2 (7)	1 (7)	1.0

Values are n (%) unless otherwise noted.
FDG-PET = 2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography; SUV = standard uptake value at FDG-PET scan; CNCB = core-needle cutting biopsy.
Deauville scale scores = 4, uptake moderately > liver; 5, uptake markedly higher than liver and/or new lesions (reference #13).

site with FDG focal uptake (DS 4 score), while 11 (73%) patients had two or more non-contiguous lymph node sites with FDG focal uptakes (DS 5 scores, 8 cases; DS 4 scores, 3 cases) and 3 (20%) patients had new lesions in extranodal sites plus lymph nodal areas showing FDG focal uptakes (DS 5 scores, 3 cases). The remaining

Table 3
Antilymphomatous therapy which each group of patients received.

Characteristics	All patients	Negative PET	Positive PET/negative biopsy	Positive PET/positive biopsy
No. of patients	169	126	28	15
ABVD alone	76 (45)	68 (54)	8 (29)	–
ABVD + cRT	78 (46)	58 (46)	20 (71)	–
ABVD plus HDCT/AHSCR	15 (9)	–	–	15 (100)

Values are n (%) unless otherwise noted. ABVD = Doxorubicin 25 mg/m^2 *IV* on days 1 and 15, bleomycin 10 IU/m^2 *IV* on days 1 and 15, vinblastine 6 mg/m^2 *IV* on days 1 and 15, dacarbazine 375 mg/m^2 *IV* on days 1 and 15 (reference #2). cRT = Consolidation radiotherapy consisted of irradiation for 30 Gy at the initial bulky area containingresidual nodes with long axis >2.0 cm detected by CT scans (references #16, #17). HDCT = High dose chemotherapy; IGEV = Ifosfamide 2000 mg/m^2 *IV* over 2 hours on days 1-4, MESNA700 mg/m^2 *IV* over 30 minutes prior to and 4 hours and 8 hours after ifosfamide on days 1–4 (three doses per day), gemcitabine 800 mg/m^2 *IV* over 30 minutes on days 1 and 4, vinorelbine 20 mg/m^2 *IV* push over 5 minutes on day 1, prednisone 100 mg *PO* on day 1-4; repeated every 3 weeks (for a maximum of 4 cycles). AHSCR = Autologous hematopoietic stem cell rescue; BEAM conditioning regimen consisted of BCNU 300 mg/m^2 *IV* day − 7, etoposide 200 mg/m^2 *IV* days − 6 to − 3, cytarabine 400 mg/m^2 days − 6 to − 3, and melphalan 140 mg/m^2 day −2; FEAM conditioning regimen consisted of fotemustine 150 mg/m m^2 *IV* days −7 and −6, etoposide 200 mg/m^2 *IV* days − 6 to − 3, cytarabine 400 mg/m^2 days − 6 to − 3, and melphalan 140 mg/m^2 day −2. Median reinfused CD34+: 3.5×10^6/kg (range 1.1–11).

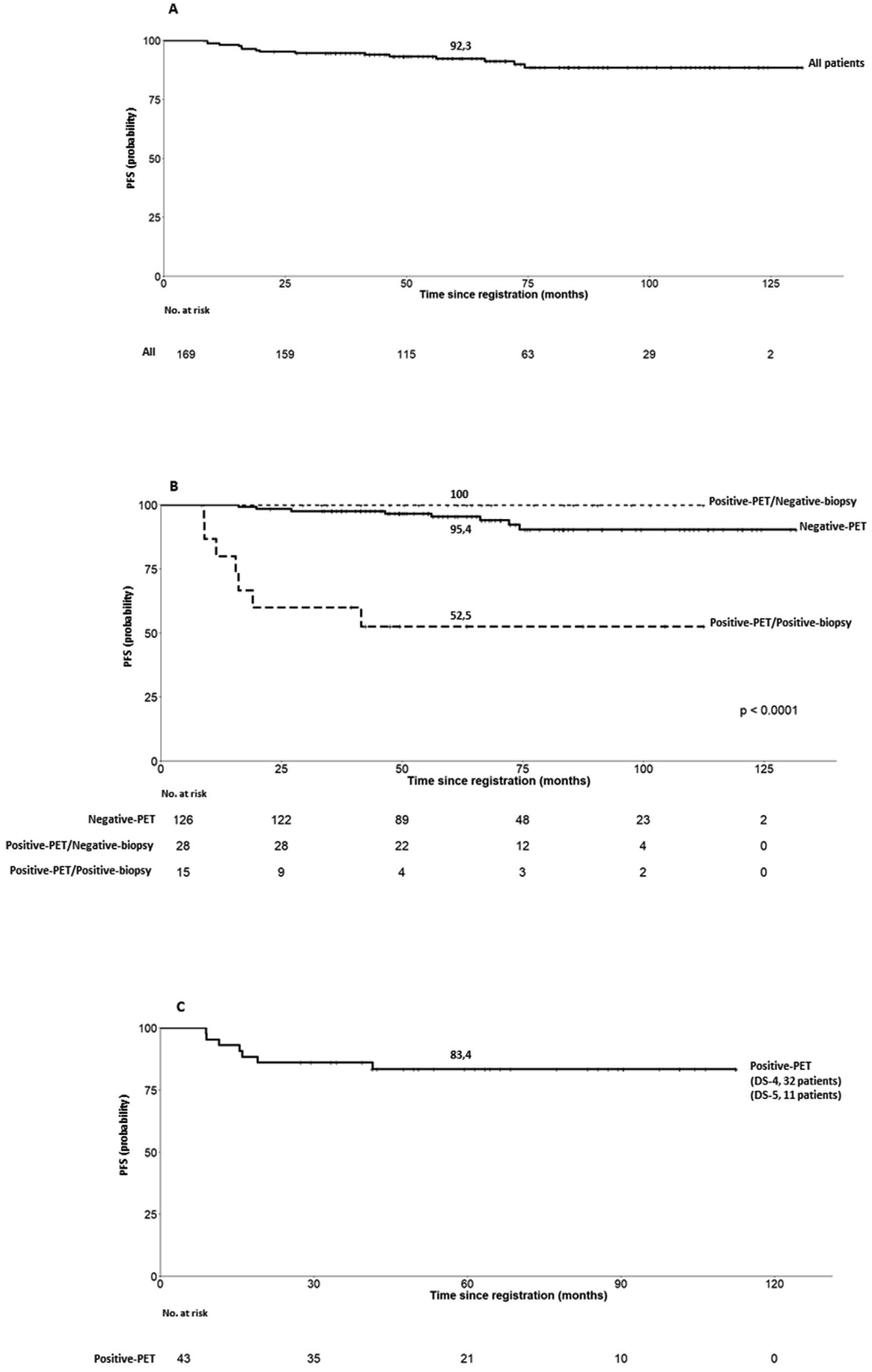

Fig. 2. Progression-free survival (PFS) showing event occurrence in patients during follow-up. PFS for all patients analysed in the study (total events, 15) (A). (B) PFS according to the restaging FDG-PET and core-needle cutting biopsy (events in the positive PET/negative biopsy group, none; events in the negative PET group, 7; events in the positive PET/positive biopsy group, 8). (C) PFS for patients with positive PET (DS 4–5 scores) at restaging. Time since registration, months from the start of ABVD. FDG-PET = 2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography.

patients, who had positive PET/negative biopsy showed FDG focal uptakes (in the category of DS 4 score) at PET scans in the following sites: 14 patients in a single lymph node site and other 14 patients in two or more non-contiguous lymph node sites.

3.4. Response-adapted strategy

After ABVD, which was administered on schedule in all patients (median dose intensity of ABVD therapy: 100% with a range of 89%–101%), according to PET combined with CNCB diagnostic work-up, 126 of 169 (74%) patients had negative PET (negative PET group), 28 of 169 (17%) patients had positive PET/negative biopsy (positive PET/negative biopsy group) and only 15 of 169 (9%) patients had biopsy-confirmed residual disease (positive PET/positive biopsy group). Table 3 shows in detail response-adapted strategy based on these findings. Antilymphomatous therapy included ABVD alone in 45% of patients, combined modality treatment with ABVD + cRT in 46% of patients and ABVD plus HDCT/AHSCR in 9% of patients.

3.5. ABVD plus HDCT/AHSCR

The 15 patients with biopsy-confirmed residual disease received salvage chemotherapy with ifosfamide, gemcitabine, vinorelbine and prednisolone for a median of 3 cycles (range, 2–4). At that point, they underwent peripheral blood stem cells harvest (average of CD34+ cells collected: 3.5×10^6 per kilogram of body weight [range of CD34+ cells collected per kilogram of body weight, $1.1–11 \times 10^6$]) followed by autologous HSC transplantation for primary refractory HL (ABVD plus HDCT/AHSCR subset, n = 15 cases).

3.6. ABVD + cRT and ABVD alone

The remaining 154 patients (91%) did not change the initially planned therapeutic strategy, having negative PET or positive PET/negative biopsy.

In particular, after ABVD program, 78 patients with negative PET (n = 58) and positive PET (all in the category of DS 4 scores)/negative biopsy (n = 20) underwent localised irradiation to initial large nodal mass sites containing residual lymphadenopathies with median of long axis diameters of 2.5 cm (range, 2.0–3.2 cm) at CT scans (ABVD + cRT subset, n = 78 cases).

By contrast, 76 patients with negative PET (n = 68) and positive PET (all in the category of DS 4 scores)/negative biopsy (n = 8) without significant residual lymphadenopathies (long axis diameter < 2 cm) at CT scans underwent follow-up and no further antineoplastic treatment after six full cycles of ABVD (ABVD alone subset, n = 76 cases).

3.7. Treatment outcome

With a median follow-up of 64.5 months (range, 8.5–131.6 months) from enrolment, the estimated 5-year PFS of the entire analysed population was 92% (95% confidence interval [CI], 86.6%–96%; Fig. 2A). Overall, there were 15 events during the follow-up: 8 events occurred in the group of patients with positive PET/positive biopsy given a PFS of 52.5% for this group, and 7 events occurred in the group of patients with negative PET given a PFS of 95.4% for this group (Fig. 2B). In particular, eight patients had persistent refractory disease, and seven patients relapsed. Of them, eight patients had received ABVD plus HDCT/AHSCR, five patients ABVD alone and two patients ABVD + cRT. No patients in the positive PET/negative biopsy group suffered from events (PFS, 100%; Fig. 2B).

The estimated 5-year OS of the entire analysed population was 96% (95% CI, 93%–99%). Seven patients died as a result of resistant or progressive disease.

3.8. Treatment toxicity

Regarding the side-effects of ABVD alone and ABVD + cRT program, twenty percent of patients had haematological toxicity of grade >2. The non-hematological toxicity (including pneumonitis, cardiovascular abnormality and peripheral neuropathy) of grade >2 was recorded in 5% of patients. No side-effect was life-threatening or fatal.

Regarding the side-effects of ABVD plus HDCT/AHSCR program, grade 3 haematological toxicities were recorded in 60% of patients and grade 4 haematological toxicities were recorded in the remaining 40% of patients. Extrahematologic adverse events (febrile neutropenia, bacterial infections) occurred in 20% of patients. No treatment toxicity–related deaths occurred.

Secondary solid tumours were recorded in only two patients (thyroid cancer in non-irradiated areas, for both cases) for the entire analysed population.

3.9. Predictive factors of positive biopsy

In searching for distinctive features in patients with CNCB-proved residual HL, we investigated clinical, laboratory and radiological findings (Table 2). Most of these patients had DS 5 scores at restaging PET: 11 of 15 (73%) with DS 5 scores versus 4 of 15 (27%) with DS 4 scores (P = 0.01). We found a statistically significant difference in the highest SUV_{max} values at biopsied sites between patients with negative biopsy (median SUV_{max} measurements, 4.5) and patients with positive biopsy (median SUV_{max} measurements, 9; P = 0.003). Patients with positive biopsy had a less lymphadenopathy mass size shrinkage after ABVD: median of residual lymph node long axis diameters was 3.5 cm in patients with positivebiopsy versus 2.5 cm in patients with negative

biopsy (P = 0.06). Moreover, patients with positive biopsy had higher values of lactate dehydrogenase in the blood: median of serum lactate dehydrogenase was 450 U/L in patients with positive biopsy versus 250 U/L in patients with negative biopsy (P = 0.04).

4. Discussion

In this study, we showed that a PET/CNCB-driven strategy was able to distinguish the responders from non-responders at the end of standard front-line chemotherapy program of advanced-stage HL. With this combined diagnostic work-up approach, we identified only a minority (9%) of patients requiring treatment intensification including salvage regimen and HDCT/AHSCR. The remaining 91% of patients after six full cycles of ABVD could safely continue the original therapeutic plan consisting of imaging interval scans with no further antineoplastic treatment or consolidating irradiation (30 Gy) on initial bulky sites (Table 3) [2]. In particular, several patients (46%) received combined modality treatment of ABVD plus localised radiation on CT scans selected sites containing residual nodal masses with long axis diameter ≥2.0 cm [16,17]. With an adequate prolonged follow-up, the final results of this trial showed a 5-year PFS of 92%, 2 points higher than that hypothesised (90%) by the study design (Fig. 2A).

To the best of our knowledge, our data strongly support for the first time the role of routine biopsy in a minority of patients with advanced HL identified by restaging PET positivity (according to the DS system) [13] and considered at risk of disease resistance or recurrence. The imaging-guided CNCB of FDG-avid residual lymph nodes was feasible in all patients and allowed for allocation to HDCT/AHSCR only in that small group of patients better identified as at the highest risk of induction treatment failure, that is, positive PET/positive biopsy. We would like to underline that in this setting the vast majority of patients (14/15, 93%) had FDG focal uptakes in multiple non-contiguous lymph nodes (n = 11 cases) or new extranodal (lung, liver or bone) lesions (n = 3 cases) making salvage treatment with radiation impractical.

Subsequently, 8 patients (53%) had an unfavourable response to HDCT/AHSCR. This subgroup clearly deserves innovative treatment approaches, including brentuximab vedotin or PD-1 blockade alone or in combination [21,22]. On the other hand, the high rate of localised irradiation in the positive PET/negative biopsy group was likely due to the large size of residual lymph nodes at the initial bulky site. In fact, 20 of the 28 (71%) patients of this group received consolidating radiotherapy on large nodal mass area owing to a relative reduction of about less than 50% of the maximal long axis diameter as measured by CT scans. Although the needle biopsies resulted negative for malignancy, the presence of metabolically active residual tissue with a not enough good shrinkage at CT scans [23] led us to leave the therapeutic strategy initially planned unaltered.

Unexpectedly, of the 43 patients with positive PET at restaging, only 15 (34.8%) had biopsy-confirming active HL [15]. The remaining 28 patients had negative biopsies (Table 2). Sampling error in these 28 cases with a positive end-of-treatment PET and negative CNCB cannot be excluded. Of them, 20 of 28 (71%) cases received consolidating irradiation on residual FDG uptakes, while only 8 of 28 (29%) underwent no further antineoplastic treatment. Thereafter, CNCB-proven negative FDG uptakes were associated with an excellent outcome: 100% of these patients showed progression free at 5 years achieving negative follow-up PET scans with the initially planned therapeutic approach. Moreover, outcomes of patients with positive PET/negative biopsy and those with negative PET were similar (5-year PFS was 100% vs. 95.4%, respectively; P = 0.18); whereas, the outcomes of patients with positive PET/positive biopsy were significantly worse than those of the other patients (5-year PFS, 52.5%; P < 0.001; Fig. 2B). In this way, we have likely cured the large majority of patients with end-of-treatment positive PET (5-years PFS of the entire positive PET group, 83.4%; Fig. 2C), correctly sparing intensified cytotoxic agent regimens in about two of three of them.

Stratifying the patients by restaging FDG-PET interpreted according to the DS [13] and the CNCB findings, for score 5 histology showed residual HL in 11 of 11 patients and for score 4 histology showed residual HL only in 4 of 32 patients. Thus, the warning of a non-malignant FDG uptake may particularly harbour in imaging scans reported as a DS score of 4 [13], which represented in our study the majority of those patients with PET positive results (32/43, 74%) after ABVD program. Of interest, the majority of histologic findings in this series was reported to be due to (probably chemotherapy induced) inflammatory changes (sarcoid-like granulomatosis; Table 2). Inflammatory tissue contains neutrophils and activated macrophages that actively accumulate FDG; therefore, it is indistinguishable from viable residual tumour at PET scans (Fig. 3) [24].

As in other reports [1,12], a negative restaging PET showed a very good diagnostic accuracy in predicting benignity. A majority (94.5%) of patients with negative restaging PET remained in sustained complete remission, indeed likely cured. However, for seven patients with DS 1–3 scores PET scanning failed to detect subclinical disease, giving a false-negative rate of 5.5%. In particular, five (5/68; 7.3%) patients who had received ABVD alone and two (2/58; 3.4%) patients who had received ABVD + cRT relapsed during the follow-up (Fig. 1). The mechanism by which persistent

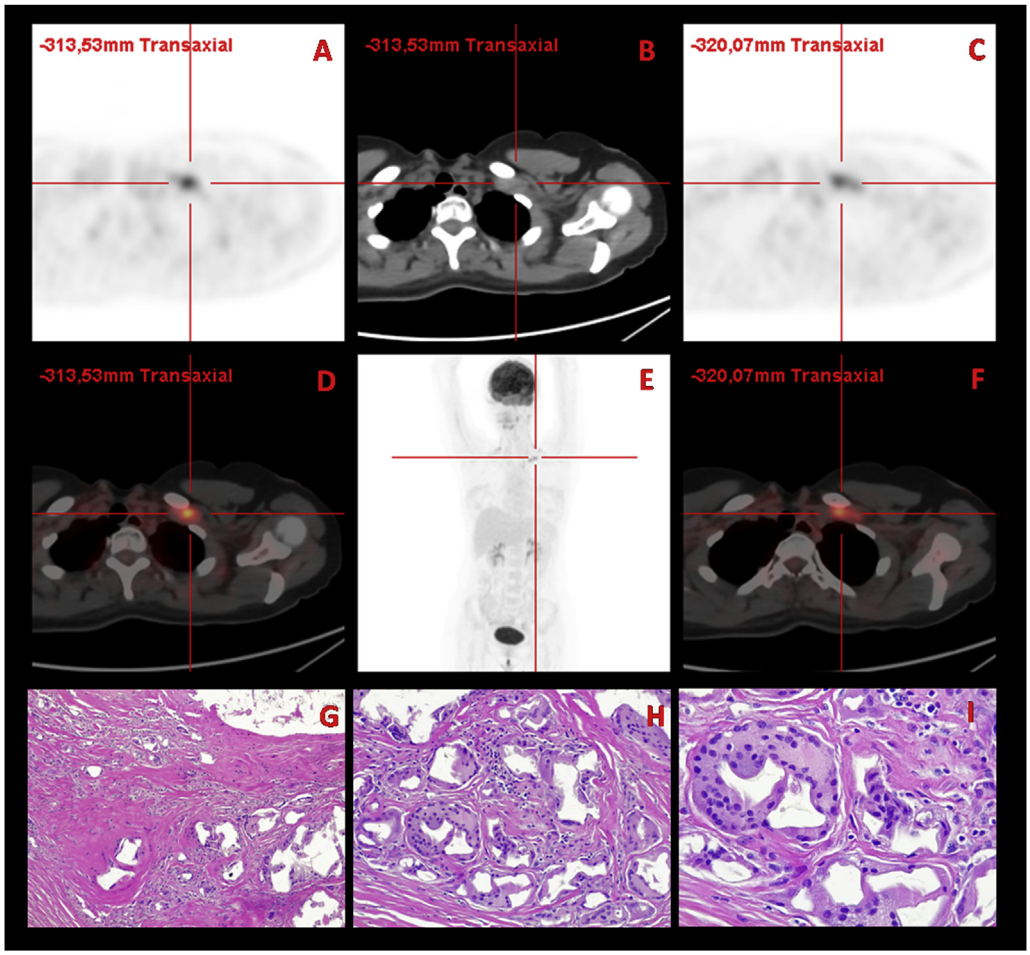

Fig. 3. Positron emission tomography/computed tomography showing 2-deoxy-2[F-18] fluoro-D-glucose-avid residual lymph nodes reported as a Deauville scale score of 4, in the left subclavicular area (A, B, C, D, E, F). Core-needle cutting biopsy findings of the above lymphadenopathy: granulomatous inflammation [haematoxylin and eosin, original magnification ×10 (G); haematoxylin and eosin, original magnification ×20 (H); haematoxylin and eosin, original magnification ×40 (I)].

lymphoma lesions may have such behaviour at PET scanning is unclear. An explanation could be that a longer time after chemotherapy is needed to recover a sufficient volume of neoplastic cell density for its detection by PET: preferably 6–8 weeks, after completion of the last chemotherapy cycle [1,12].

Recently, another approach using FDG-PET imaging scans has been proposed in advanced HL. In this case, response-adapted strategy is based on PET performed early during induction therapy. There are important randomised controlled trials pointing out the advantage of changing therapy after two cycles of ABVD, thus leading to the use of doxorubicin, vinblastine and dacarbazine instead of ABVD to decrease toxicity in case of negative interim (after 2 cycles of ABVD) PET [25] and escalated bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone intensification in case of positive interim PET [26].

Our study suffers from several major limitations. First, this trial was conducted in one single centre. Therefore, studies from other institutions are needed to largely assess (1) interobserver and interequipment imaging variability, in particular FDG-PET interpretation according to the Deauville 5-point scale [1,12,13]; (2) core-needle specimen quality reproducibility, for example, tissue harvested, size and preservation [14] and (3) concordance by pathologists in diagnosing lymphoma on core-needle material [15]. Second, a bias error could have been committed due to a more accurate selection of nodal target to be biopsied in the core-needle subgroup leading the study toward a higher sensitivity for the miniinvasive biopsy procedure. The presence of FDG-avid tissue particularly stiffened and/or seated in a particularly hindered region could lead to the sampling error of CNCB, suggesting that in some instances, there is a need for traditional surgical biopsy for the correct histological assessment of lymph nodes [15]. Third, this trial also included patients with stage II disease, which usually has a better outcome than stage III-IV disease [2]. Fourth, in this study 58 of the 126 (46%) patients [stage II, 38 (65%) cases; stage III-IV, 20 (35%) cases]

with restaging negative PET were given radiation after six full cycles of ABVD. We acknowledge that these patients may not have required radiation. According to the European Society for Medical Oncology clinical practice guidelines for diagnosis, treatment and follow-up of HL, the question of whether cRT can be safely omitted in patients with advanced HL who have residual masses at the end of chemotherapy has not yet been definitively answered [2]. In our experience, based on the results of a randomised trial using a less conventional anthracycline-based chemotherapy regimen of vinblastine, etoposide, bleomycin, epirubicin and prednisone [16], as well as in the experience of the authors of National Comprehensive Cancer Network clinical practice guidelines in oncology [27], the addition of radiotherapy is suggested to residual lymphadenopathies with FDG uptake of DS $\leq$ 3 scores in initial bulky areas or selected PET + sites. In the last decade, radiotherapy dose and volumes have significantly decreased, thereby decreasing toxicity risks [2,28,29]. However, recent and exhaustive literature shows that radiation in such instances does not add a significant increase in PFS [23,26]. Finally, larger prospective trials are needed to show if the ABVD program [2]may be potent enough to eradicate all Hodgkin's-Reed Sternberg cells in residual nodes with core-needle biopsy–proven non-viable-tumour–related positivity at FDG-PET scans, omitting irradiation.

Applying intensified therapies to the entire group of patients with end-of-treatment positive PET should be at least questionable, in cases of FDG-avid lymphomas. The major disadvantage could be overtreatment of a certain number of patients with positive PET who could benefit by not altering the initially planned therapeutic strategy [2]. Until now, there are no relevant data on the systematic histopathology verification of the metabolically active residual tissue at the end of ABVD restaging [7] even in studies that use the most recently introduced international (Deauville/Lugano) criteria [1,12,13]. With optimal conditions and experienced physicians, imaging-guided CNCB enabled safe and effective histological assessment in a significant sample of patients with HL with residual FDG-avid lymph nodes. Noteworthy, US (a cheaper imaging tool for the diagnostic work-up of HL, as already described by us) [20,30] was successfully used in the most patients of our series (Table 2). Based on our results, the current guidelines (using DS of 4 score as cut-off value) [1,12,13] result in a

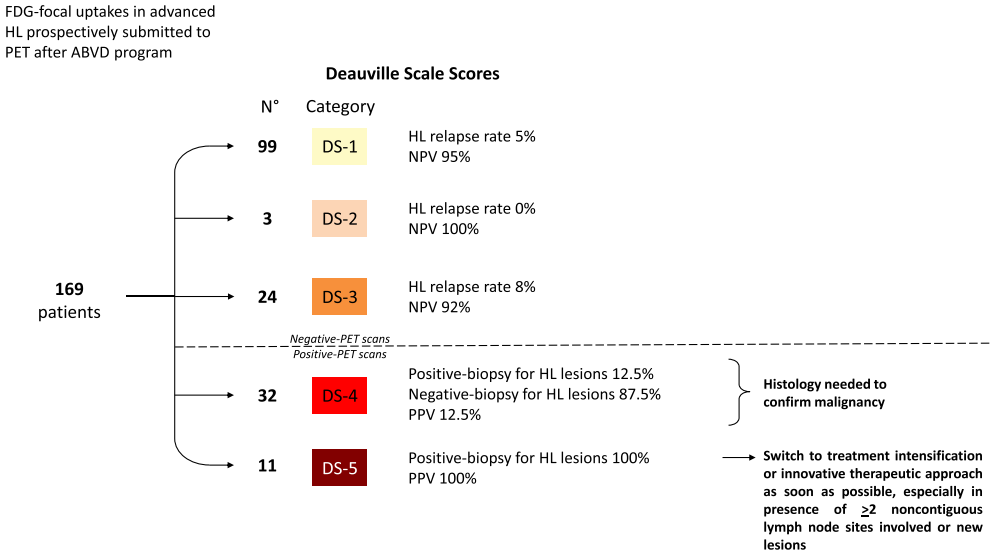

Fig. 4. FDG focal uptakes in advanced HL prospectively submitted to PET after ABVD program. In scans classified as Deauville scale (DS) 1–3 scores, the reference standard for determining the negative predictive value (NPV) of restaging FDG-PET was progression-free survival (PFS). The NPV of DS in this group of patients with the end of ABVD negative PET (DS $\leq$ 3 scores) should be evaluated considering that 46% of them received consolidation radiotherapy after restaging (refer text). For the scans classified as DS 4–5 scores, the reference standard for assessing the positive predictive value (PPV) of restaging FDG-PET was the histology obtained by imaging-guided core-needle cutting biopsy. The PPV of DS in this group of patients with the end of ABVD positive PET (DS $\geq$ 4) should be evaluated considering that 71% of patients in the positive PET/negative biopsy group received consolidation radiotherapy after restaging (refer text). NPV = negative predictive value is the probability that subjects with a negative screening test truly do not have the disease (number of true negatives/number of true negatives + number of false negatives). PPV= positive predictive value is the probability that subjects with a positive screening test truly have the disease (number of true positives/number of true positives + number of false positives). FDG = 2-deoxy-2[F-18] fluoro-D-glucose; HL = Hodgkin lymphoma; PET = positron emission tomography. ABVD = 25 mg/m^2 of doxorubicin I.V. on days 1 and 15, 10 IU/m^2 of bleomycin I.V. on days 1 and 15, 6 mg/m^2 of vinblastine I.V. on days 1 and 15 and 375 mg/m^2 of dacarbazine I.V. on days 1 and 15 (reference #2). I.V. = intravenous.

certain negative histology rate and should hence be improved (Fig. 4). Outside of a clinical trial, we suggest biopsy confirmation of DS 4 (FDG focal uptakes moderately > liver) [13] score before initiating a change in planned therapeutic strategy, in particular cRT omission [16,27]. By contrast, the DS 5 (FDG focal uptakes markedly higher than liver and/or new lesions) [1,12,13] score showed consistently positive histology and was associated with unsuccessful conventional therapy, promptly requiring treatment intensification or innovative therapeutic approaches [2,3,21,22].

Conflict of interest statement

Authors have no relevant financial conflict of interest to declare.

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