MedPrecis
Never Stop Exploring

Journal of Clinical Oncology and Cancer Research
Volume 1, Issue 1

Research Article | Open Access

Identifying and Consequences of Spread Cancer Cells in Non-Small Cell Lung Cancer Patients

Harlos Stein[1*], Ernst Morgan[2], Rodrigues Wright[2]

[1]Department of Medical Oncology and Hematology, Cancer Care Manitoba, and Department of Internal Medicine, University of Manitoba, Winnipeg, Canada

[2]Department of Oncology, Juravinski Cancer Centre, Hamilton, Canada

***Corresponding Author:** Harlos Stein, Department of Medical Oncology and Hematology, CancerCare Manitoba, and Department of Internal Medicine, University of Manitoba, Winnipeg, Canada. **E-mail:** harlos_s@gmail.com

Received date: January 19, 2023; **Accepted date:** February 15, 2023; **Published date:** February 21, 2023

Citation: Stein H, Morgan E, Wright R. Identifying and Consequences of Spread Cancer Cells in Non-Small Cell Lung Cancer Patients. J Clin Oncol Cancer Res . 2023;1(1):1-7.

ABSTRACT

Introduction: Evidence indicates that disseminated neoplasm cells (DOCs) in the bone marrow (BM) serve as precursors to distant metastases in cancer. Identifying DOCs in non-small cell carcinoma (NSCLC) is crucial for understanding metastatic potential and uncovering potential treatment targets.

Study Objective: This study aims to assess the detection likelihood of DOCs in BM, determine the frequency of BM involvement in NSCLC patients, and evaluate their impact on bone marrow lymphocyte populations.

Methods: Sixty-two BM samples from NSCLC patients were analyzed using morphological and immunological techniques. Flow cytometry (FACS Canto II, USA, Kaluza Analysis v2.1 software) was employed, utilizing antibodies to CD45 and cytokeratins labeled with various fluorochromes.

Results: DOCs (EPCAM+CD45-) were identified in 43.5% of patients (threshold: one cell per ten million myoelectricities). CD133 expression (CD133+EPCAM+CD45-) was present in 23.3% (9/27) of cases. DOC presence did not correlate with tumor size, lymph node status, or tumor stage. Highest DOC detection rates were in stages Ia and IIa (60.7% and 58.3% respectively). BM involvement was observed in 45% of adenocarcinoma cases and 37% of epithelial cell carcinoma cases (p=0.501). DOCs were more prevalent in well-differentiated tumors (p=0.023). No significant correlations were found with X-ray parameters. A 4% reduction in white blood cell precursors was noted with 4% BM involvement (p=0.036).

Conclusion: This study establishes the presence of DOCs in the BM of NSCLC patients, with a 43.5% BM

involvement rate. DOCs are detectable even in early NSCLC stages. BM involvement correlates with tumor differentiation, being more frequent in adenocarcinoma compared to epithelial cell carcinoma of the lung. Understanding these dynamics provides valuable insights into NSCLC metastasis and potential therapeutic avenues.

Keywords: Bone marrow; Non-small cell carcinoma; Morphology; Flow cytometry; Cancer stem cells.

INTRODUCTION

Non-small cell carcinoma (NSCLC) stands as the leading cause of death from malignant tumors in most countries globally. Despite significant strides in drug therapy based on the tumor's molecular biology, the survival rate for NSCLC patients remains dishearteningly low, with only 18% of patients experiencing a 5-year survival [1].

Presently, scientific efforts are concentrated on developing methods to enhance the effectiveness of antineoplastic immunologic responses. A substantial portion of research delves into understanding the immuno-biological mechanisms of hematogenous metastasis, with a focus on disseminated neoplasm cells (DTC) frequently found in the bone marrow (BM) of patients [5-8]. Examining BM is crucial as DTC residing there interact with a new microenvironment, dictating their fate. While some DTC may remain dormant, others evade immune responses, leading to the formation of secondary tumors. These cells establish a pathological niche, disrupting controlled cellular [10] and molecular mechanisms of the microenvironment [9-11]. The BM becomes a sanctuary for DTC, enabling their uncontrolled growth and progression [2,12]. Recent studies have revealed the diversity of DTC, expressing various surface antigens [7], distinguishing them from the primary neoplasm and reflecting the intricate relationship between DTC and their microenvironment. Evidence suggests that DTC in the BM may possess genomic profiles distinct from the primary neoplasm [13]. This adaptability grants them traits essential for survival, akin to cancer stem cells (CSCs), playing a pivotal role in self-maintenance and metastasis, marked by dysregulated signal pathways and aberrant phenotypes.

Modern techniques enable sensitive DTC detection, providing essential insights into metastatic mechanisms and aiding in comprehending molecular changes underlying drug resistance. Consequently, DTC are considered potential targets for drug therapy. Addressing these pathological seeds before metastasis emerges has gained attention, leading to the exploration of regimens preventing DTC activation and proliferation, showing promise in specific cancer types.

This study aims to evaluate DTC detection in BM among NSCLC patients and explore the frequency of BM lesions, correlating them with clinical and morphological neoplasm parameters. Through this research, we anticipate gaining a deeper understanding of neoplasm growth patterns, facilitating the identification of new targets for drug therapy.

MATERIALS AND METHODS

This study involved a cohort of sixty-two patients diagnosed with non-small cell lung cancer (NSCLC) who received treatment at the Federal State Budgetary Institution Research Center for Medical Radiology named after N.N. Blokhin, under the Ministry of Health of Russia. The research was conducted in accordance with the guidelines of the institution's ethical committees (Local Ethics Committee of N.N. Blokhin Russian Cancer Research Center, Ministry of Health of the Russian Federation; UDC 616-006, Reg. AAAA-A16-116122210071-4, Inv. 479) and obtained the informed consent of all participating patients.

The patients' ages ranged from seventeen to eighty years, with an average age of sixty-three years. The study predominantly included male patients, constituting forty-eight individuals (77.4%), while the number of female patients was fourteen (22.6%). All patients underwent surgical interventions, except for one case in which a diagnostic biopsy was performed.

Based on the pathological findings, twenty-seven patients (43.5%) were diagnosed with squamous cell carcinoma, thirty-three patients (53.3%) had adenocarcinoma, and two cases (3.2%) were diagnosed with other histological forms. Among the tumors, fifty percent (n = 31) were moderately differentiated (G2), six-point-five percent (n = 4) were well-differentiated (G1), and a low degree of differentiation (G3) was observed in thirty-two percent (n = 20) of cases. Notably, seven cases did not have a defined differentiation grade (Figure 1).

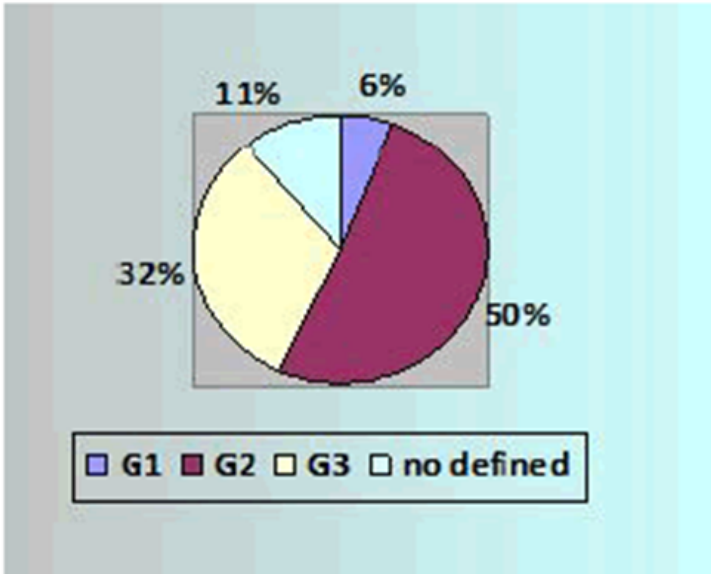

Figure 1: The degree of tumor differentiation.

Bone marrow (BM) samples were obtained through bone marrow puncture. The puncture volume did not exceed 1.0 ml to prevent dilution with peripheral blood, which could compromise the sample quality. The study of BM involved two methods: morphological analysis and immunological assessment [14].

For morphological analysis, six glass slides were stained using the Romanovsky methodology. X-ray counts and the search for tumor cells were simultaneously conducted by two morphologists. Immunological assessment of the presence of Disseminated Tumor Cells (DTC) in BM was performed using antibodies to cytokeratins EPCAM (Becton Dickinson, USA) and KL-1 (Immunotech, France), CD133 (Becton Dickinson, USA), and CD45 (Becton Dickinson, USA), directly labeled with various fluorochromes: FITC, PE, V500, V450, PerCP. Cell sorting was conducted using a FACS Canto II flow cytometer, USA. The results were analyzed using the Kaluza Analysis v2.1 software (Beckman Coulter, USA). Approximately twenty million myelocaryocytes (or all cells from the sample) were evaluated. The presence of Disseminated Tumor Cells (DTC) was identified by the lack of CD45 expression in conjunction with the expression of EPCAM or KL-1. Statistical analysis was performed using IBM-SPSS Statistics v software.

RESULTS AND DISCUSSION

Morphological analysis of the bone marrow (BM) involved the calculation of myelograms and the search for tumor cells. Morphologically, Disseminated Tumor Cells (DTC) in BM were detected in only one out of sixty-two cases [15]. In the immunological assessment of DTC presence, an intensity of one neoplasm cell per ten million myelokaryocytes was considered. DTC (CD45-EPCAM+) were found in 43.5% of the BM samples (n = 27) (Figure 2).

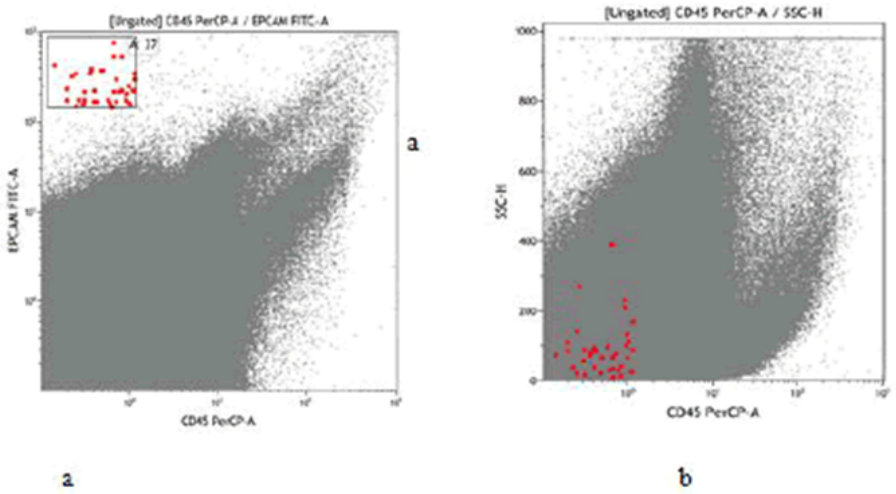

Figure 2: Identification of disseminated tumor cells in the BM of a patient with NSCLC by flow cytometry.

In nine patients (14%), a single tumor cell was detected among ten million myelocaryocytes in the bone marrow (BM) samples. In other cases, the number of tumor cells varied from 2 to 242. A distinct cluster of tumor cells (micrometastasis) was diagnosed in 2 (3.2%) out of the sixty-two patients with non-small cell lung cancer (NSCLC). The highest number of Disseminated Tumor Cells (DTC) in BM was found in a patient with stage IIA (T2aN1M0), squamous cell carcinoma (Figure 3). Interestingly, these two patients did not have micro-metastases identified through morphological methods.

Disseminated Tumor Cells (DTC) were detected in four BM samples (6.65%) using the threshold level of one cell per one million myelokaryocytes. Notably, stage II NSCLC was identified in two cases, comprising both adenocarcinoma and squamous cell carcinoma. The presence of DTC in BM was confirmed in one case using the threshold level of one cell per one hundred thousand myelokaryocytes, observed in a patient with stage IIA (T2aN1M0).

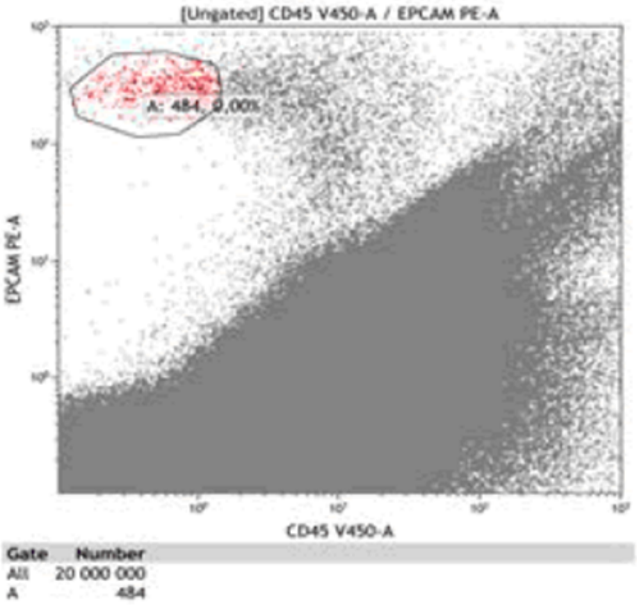

Figure 3: Disseminated tumor cells in the BM of patient J. This figure shows an example of the DTC detection in NSCLC as a cluster.

It is important to mention that Cancer Stem Cells (CSCs), which exhibit high resistance to conventional cancer therapies, play a significant role in metastasis. These cells are characterized by abnormal activation of signaling pathways, leading to uncontrolled cell proliferation and maintaining their stem-like properties. In our study, CSCs within NSCLC DTCs were identified by the expression of CD133. CD133 expression was analyzed in twenty-seven BM samples, revealing that nine samples (33.3%) contained CD133+EPCAM+CD45- cells. The number of DTCs expressing CD133 in these samples ranged from 1 to 5 cells (Figure 4).

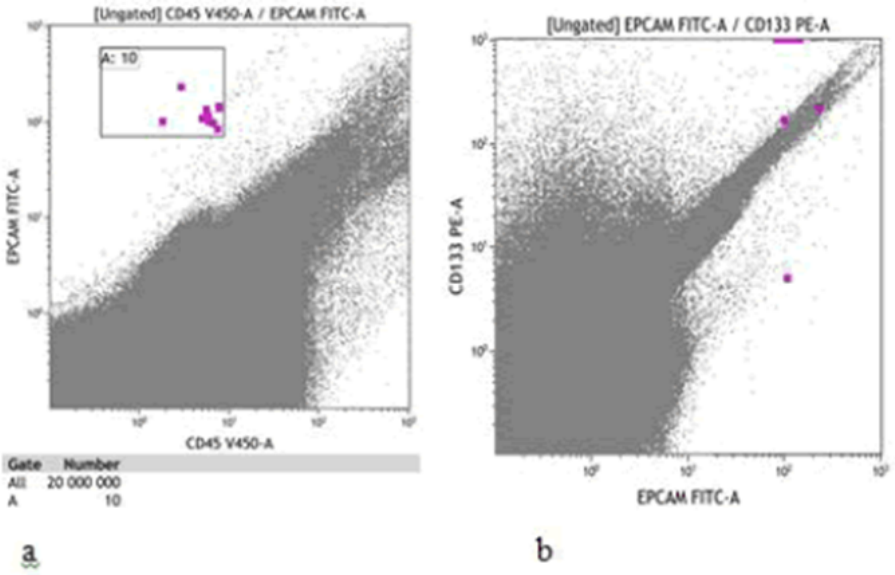

Figure 4: Identification of CD133+EPCAM+CD45- in the BM of a patient with NSCLC by flow cytometry.

The study explored the presence of Disseminated Tumor Cells (DTC) in bone marrow (BM) samples, correlating it with various clinical and morphological factors in patients with non-small cell lung cancer (NSCLC) [16]. The research established that DTC was present in 6.65% of BM samples at a threshold of one cell per ten million myelocaryocytes. Notably, there was no significant association between the presence of DTC and the patient's age or sex.

The analysis focused on the relationship between BM lesions and the clinical and morphological characteristics of the primary tumor. DTCs were found even in the early stages, suggesting an early hematogenous micrometastasis process. The highest detection rates of DTC were in stages IIA and IIB, amounting to 60.7% and 58.3%, respectively.

Further examination considered the primary tumor's size and the number of affected lymph nodes concerning the presence of DTC in BM. While no reliable dependency was found concerning tumor size, DTC-positive cases were more prevalent at stage T1 (71.4%) and less frequent as tumor size increased (36.4% and 28.6% for T3 and T4, respectively).

Moreover, the study analyzed BM lesions in relation to the tumor's histological type, revealing a higher incidence of DTC in adenocarcinoma (45.5%) compared to squamous cell carcinoma (37%). Notably, a significant association was observed between DTC presence and tumor differentiation level, with a higher detection rate in well-differentiated cancers (G1-G2, 32%) compared to poorly differentiated ones (G3-G4, 4%).

The study also explored BM parameters, indicating lower cellularity in cases with DTC presence. Additionally, variations in the leuko-erythronormoblastic ratio (LER) were noted, albeit not statistically significant. Promyelocytes were reduced in 72% of patients with DTC, compared to 84.4% without DTC. The white blood

cell maturation index was also decreased in DTC-positive cases (68%).

Concerning erythrocytopoiesis, changes in polychromatophilic forms and an increase in oxyphilic normoblasts were observed in DTC-positive cases. The study showed a tendency to modify the BM microenvironment due to DTC presence, suggesting that DTC might influence the hematopoietic system.

In summary, the research highlighted the complexity of DTC interactions with BM and indicated the need for further investigation, especially in larger cohorts. The persistence of DTC in BM, possibly due to specific molecular genetic mechanisms and the heterogeneous nature of DTC populations, underscores the intricate dynamics between tumor cells and the host microenvironment. Further studies are crucial to comprehensively understand these interactions, especially in the context of early-stage NSCLC patients.

CONCLUSION

The identification of Disseminated Tumor Cells (DTC) and understanding the risks associated with their characterization provide crucial insights into metastatic mechanisms and enhance our understanding of the changes underlying drug resistance in cancer. Given the evolving landscape of non-small cell lung cancer (NSCLC) therapy, the analysis of DTC in bone marrow (BM) and their interactions with immune cells has become a focus of intensive research.

In this study, we successfully identified DTC in patients with NSCLC. Detection of BM lesions was based on the absence of CD45 expression coupled with the presence of EPCAM or KL-1 expression. The detection rate of DTC in NSCLC patients was 43.5%, a figure comparable to DTC detection rates in other types of tumors. Notably, this rate was higher in the early stages of the disease, indicating that hematogenous micrometastasis is an early event in NSCLC progression. The observation of CD133 expression highlights the heterogeneity of DTCs and the intricate hierarchical relationships between primary tumor cells and DTCs. Furthermore, the incidence of DTC was higher in patients with adenocarcinoma, establishing a clear association between BM lesions and the degree of tumor differentiation. These findings are intriguing and warrant further investigation to assess the prognostic value of DTC in NSCLC and explore potential therapeutic interventions.

The documented relationship between BM lesions and an unfavorable prognosis across various tumors, leading to poor overall and relapse-free survival, has spurred scientists to develop methods aimed at preventing the activation and proliferation of DTC. Consequently, DTCs are considered promising targets for drug therapy, and NSCLC is no exception to this paradigm shift in cancer treatment strategies. The ongoing research in this area holds the potential to revolutionize NSCLC therapy and improve outcomes for patients in the future.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

None.

REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical Features of Patients Infected With 2019 Novel Coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.

2. Hui David S, I Azhar E, Madani TA, Ntoumi F, Kock R, Dar O, et al. The continuing 2019-nCoV epidemic

threat of novel coronaviruses to global health: The latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis. 2020;91:264-266.

3. www.who.int/docs/default-source/coronaviruse/situation-reports/20200311-sitrep-51-covid-19.pdf?sfvrsn=1ba62e57_10

4. Chan Jasper F, To KK, Tse H, Jin DY, Yuen KY. Interspecies transmission and emergence of novel viruses: Lessons from bats and birds. Trend Microbiol. 2013;21(10):544-555.

5. Zhu Na, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. New England J Med. 2020;382(8):727-733.

6. Chen Yu, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J Med Virol. 92(4):418-423.

7. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. JAMA. 2020;323(11):pp:1061.

8. Ai T. Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiol. 2020;296(2):E32-E40.

9. Fang, Yicheng. Sensitivity of Chest CT for COVID-19: Comparison to RT-PCR. Radiol. 296(2):E115-E117.

10. Waller JV. The Limited Sensitivity of Chest Computed Tomography Relative to Reverse Transcription Polymerase Chain Reaction for Severe Acute Respiratory Syndrome Coronavirus-2 Infection: A Systematic Review on COVID-19 Diagnostics. Invest Radiol. 2020.

11. Wang. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA. 2020;323(18):1843-1844.

12. Chan. Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated In Vitro and with Clinical Specimens. J Clin Microbiol. 2020;58(5):310-320.

13. Yip. Development of a Novel, Genome Subtraction-Derived, SARS-CoV-2-Specific COVID-19-nsp2 Real-Time RT-PCR Assay and Its Evaluation Using Clinical Specimens. Int J Mol Sci. 2020;21(7):pp:2574.

14. Yan C. Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. Clin Microbiol Infect Dis. 2020;26(6):773-779.

15. Long. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020;26(6):845-848.

16. Li Zhengtu. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol. 2020.