CASE REPORT

Flow Cytometry in Diagnosis of Myelomatous Pleural Effusion: A Case Report

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Abstract Plasma cell myeloma is a multifocal plasma cell neoplasm associated with increased monoclonal protein in serum and/or urine. Pleural effusions in patients with myeloma are uncommon (6 %). However, effusions due to direct infiltration of the pleura by plasma cells (myelomatous pleural effusion) are extremely rare (<1 %) and usually seen with IgA myeloma. The diagnosis of such cases requires pleural fluid cytology, electrophoresis or pleural biopsy. We present a case of myelomatous pleural effusion diagnosed using flow cytometry immunophenotyping in addition to the pleural fluid cytology. A 45 year old female was diagnosed as plasma cell myeloma (IgG kappa) in 2007. She received multiple lines of therapy during the course of her treatment including thalidomide, dexamethasone, lenalidomide, bortezomib, and doxorubicin based regimens. However, the patient had progressive extramedullary disease and developed pleural effusion in 2014. Cytological examination of the pleural fluid showed degenerative changes. Few preserved areas showed mononuclear cells including morphologically abnormal plasma cells. Immunophenotyping of these cells by flow cytometry revealed a pattern indicating neoplastic plasma cells. There was expression of CD38, CD138, and CD56, with absence of CD19, CD10 and CD45. This confirmed the diagnosis of myelomatous pleural

effusion. Subsequently, the patient was offered a dexamethasone, cyclophosphamide, etoposide and cisplatin based regimen but, she declined further treatment and succumbed to her disease 3 months later. Myelomatous pleural effusion is a rare complication of plasma cell myeloma. Flow cytometry can be used as an adjunctive technique in its diagnosis particularly in cases with equivocal cytology and electrophoresis findings.

Keywords Myelomatous pleural effusion · Complications of multiple myeloma · Flow cytometry in myeloma · Neoplastic plasma cells

Introduction

Plasma cell myeloma is a multifocal plasma cell neoplasm associated with increased monoclonal protein in serum and/or urine. Its spectrum ranges from indolent to aggressive forms. Pleural effusions in patients with myeloma are uncommon (6 %) [1]. However, effusions due to direct infiltration of the pleura by plasma cells (Myelomatous pleural effusion) are extremely rare (<1 %) [1] and more common with IgA myeloma [2, 3]. The diagnosis of such cases requires either detection of atypical plasma cells on pleural fluid cytology, demonstration of monoclonal protein on pleural fluid electrophoresis or histological confirmation on pleural biopsy. Currently multiparameter flow cytometry is being increasingly used for diagnostic characterization of neoplastic cells as well as for monitoring therapy in cases of multiple myeloma. However single case report has used immunophenotyping by flow cytometry for the diagnosis of myelomatous pleural effusion [4]. Bivariate analysis of cytoplasmic immunoglobulins and DNA content by flow cytometry was performed in other

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studies [5, 6] showing 10 % false negative result due to non specific light chain staining further emphasizing the importance of immunophenotyping along with cytological examination for confirmation of diagnosis [6]. We report a case of pleural effusion in a patient with progressive extramedullary plasma cell myeloma which was confirmed as myelomatous pleural effusion with the help of flow cytometry based immunophenotyping. The application of flow cytometry in identification and differentiation of neoplastic plasma cells from the reactive cells has also been briefly discussed.

Case Report

A 45 year old female was diagnosed as IgG kappa plasma cell myeloma at a peripheral centre in 2007. The bone marrow had 28 % abnormal plasma cells. Serum electrophoresis and immunofixation showed M-spike (level not available) with IgG kappa monoclonal protein. She also had multiple osteolytic and osteoblastic lesions in skull bones, vertebrae, multiple ribs, pelvic bones and upper third of trochanter. She was treated with thalidomide and dexamethasone based regimen there and then referred to our centre for further management. On her evaluation here, she was found to be in complete remission with absence of M-spike in serum and urine. The patient was advised high dose chemotherapy with autologous stem cell transplantation, but was reluctant. She was put on thalidomide maintenance (100 mg/day reduced to 50 mg/day later due to peripheral neuropathy) and zoledronic acid (once in 3 months). Skeletal survey repeated in 2012 showed new lytic lesions on the head of left humerus, cortical thinning of shaft of right femur and sclerotic lesions in iliac bone. On serum electrophoresis, M-protein showed increasing trend with levels reaching up to 21 g/L. She then received 4 cycles of lenalidomide (25 mg on day 1–21) and dexamethasone (40 mg weekly) every 28 days with monthly zoledronic acid, following which she achieved partial response with M-protein being 4 g/L. However, 3 months later, she developed pelvic mass in left sacral ala and iliac bone and was given palliative radiotherapy (20 Gy in 5 Gy fractions). The M-protein increased to 10 g/L. The therapy was changed to VRD regimen containing bortezomib (1.3 mg/m² on days 1, 8 and 15), lenalidomide (10 mg for 14 days) and dexamethasone (40 mg weekly) following three cycles of which she attained complete response (M-spike negative) and was put on maintenance doses of bortezomib (day 1 and 15), lenalidomide (5 mg for 14 days) and dexamethasone (20 mg weekly). In 2014, she developed abdominal lymphadenopathy. Ultrasound abdomen and pelvis revealed multiple enlarged paraaortic and aortocaval peripelvic lymph nodes with bilateral hydronephrosis. An ultrasound

guided biopsy done from the lymph nodes showed sheets of mature and immature plasma cells. The serum M-protein was now 4 g/L. Her therapy was changed to PAD regimen containing bortezomib (day 1, 8, 15 and 22), doxorubicin (30 mg/m² on day 4) and dexamethasone (40 mg weekly). She received three cycles of the same and serum M-protein became negative. One month later, the patient developed fever, generalized weakness, shortness of breath, dysphagia and persistent diarrhoea. On general examination, she had pallor, pedal edema and thickened perineal area. The systemic examination revealed right lower lobe pneumonia with synpneumonic pleural effusion. CT chest and abdomen confirmed right pleural effusion with collapse and consolidation, conglomerate lymph nodal mass in abdomen, right renal mass- likely myelomatous deposit, rectal wall thickening along with gluteal mass. A complete blood count revealed hemoglobin of 63 g/L, total white blood cell count of 5×10^9 /L, absolute neutrophil count of 3.5×10^9 /L, and platelet count of 34×10^9 /L. There were no circulating plasma cells or other abnormal cells. Bone marrow aspirate had 1 % plasma cells. However on serum electrophoresis a dense M-band (6 g/L) was obtained. Blood chemistry tests showed serum total protein- 46 g/L, albumin- 28 g/L, β_2 microglobulin >12.6 mg/L, lactate dehydrogenase- 501 U/L. Serum calcium, creatinine, uric acid, thyroid stimulating hormone and liver enzymes were within normal limits. Serology for HIV, hepatitis B and hepatitis C was negative. CMV PCR was also negative.

On thoracocentesis, hemorrhagic fluid was obtained from the pleural effusion. Pleural fluid chemistry revealed total protein—24 g/L; glucose- 94 mg/dl; adenosine deaminase was raised—120 IU/L (<35 IU/L). Pleural fluid Gram stain, fungal and acid fast bacilli smears were negative. Its cultures were sterile. Cytological examination done by cytopspin preparation of the pleural fluid revealed degenerative changes. Few preserved areas showed mononuclear cells including morphologically abnormal plasma cells with eccentrically placed pleomorphic nucleus with open chromatin, prominent nucleoli having moderate amount of basophilic cytoplasm (Fig. 1).

Immunophenotyping by flow cytometry was also performed using the FC-500 (Beckman Coulter, USA). Two tubes were processed- CD38/CD10/CD19/CD138/CD45 and CD38/CD19/CD56/CD45. CD138 could not be included in the second tube since both CD138 and CD56 were labelled with the same fluorochromes. The primary gating of the events was done using CD38/CD138 in the first tube and CD38/CD45 in the second tube. The gated cells were around 75 % of all cells and showed expression of CD38, CD138, and aberrant expression of CD56 (moderate to bright), with absence of CD19, CD10 and CD45 (Fig. 2). Thus, immunophenotyping of these cells revealed a pattern

Fig. 1 Pleural fluid cytospin preparation showing mononuclear cells including morphologically abnormal plasma cells (Jenner-Giemsa $\times 1000$)

indicating neoplastic plasma cells with aberrant expression of CD56 and lack of CD19.

The patient refused a dexamethasone, cyclophosphamide, etoposide and cisplatin (DCEP) based regimen. She was prescribed metronomic therapy using cyclophosphamide (50 mg for 21 days), prednisolone (50 mg for 7 days followed by 20 mg on alternate days for 2 weeks) and thalidomide (100 mg daily) based oral therapy and discharged on request. She died of progressive disease at home, 3 months later.

Discussion

Pleural effusion in multiple myeloma patients may be due to various etiologies including congestive heart failure due to amyloidosis, nephritic syndrome, chronic renal failure, hypoalbuminemia, pulmonary emboli, infection, secondary neoplasm or pleural myelomatous involvement [1, 7]. Myelomatous pleural effusion is rare [1, 8]. The pathogenesis of such phenomenon may be due to direct extension or implantation of tumor deposits on the pleura or lymphatic obstruction from mediastinal lymph node infiltration. [1]. The myelomatous pleural effusions are most commonly reported in cases with IgA myeloma [2, 3]. A recent single centre experience of 23 patients with myelomatous pleural effusion had majority of the patients with IgA type followed by lambda light chain myeloma and IgG myeloma [3]. Another compilation of case reports showed that majority of cases with pleural effusion as initial manifestation of myeloma had IgG myeloma [9]. Our patient had IgG myeloma but the pleural effusion occurred as a late complication rather than at presentation.

Another finding we would like to highlight is that of raised ADA level in the pleural fluid. Raised ADA levels are mostly associated with tubercular pleural effusions, [10] however, have been noted in some other benign (e.g. pneumonia, rheumatoid arthritis, asbestos pleurisy) and malignant conditions (e.g. lung cancer, mesothelioma, lymphoma, hepatoma etc.) [11, 12]. Raised ADA levels

Fig. 2 Flow cytometric analysis of the plasma cells in pleural effusion—Gating of plasma cells using CD38/CD138 in the first tube (a), CD19 negativity in the gated population (b), Gating of plasma cells using CD38/CD45 in the second tube (c), CD56 positivity on gated cells (d)

have been reported in myelomatous pleural effusion as well [9, 13–15]. It has been postulated earlier that the raised ADA levels may reflect activation of the immune system, and that is why such cases also commonly have enlarged lymph nodes [9]. Our case also had high level of pleural fluid ADA and enlarged lymph nodes which may be due to the active immune response. Myelomatous pleural effusion can be diagnosed with the help of pleural fluid cytology, pleural fluid electrophoresis or pleural fluid biopsy [2]. Abnormal karyotype on cytogenetic analysis in the pleural fluid can also provide unequivocal evidence of malignancy [5] however, it is not always possible. It is also important to distinguish neoplastic and reactive plasma cells. The reactive plasma cells in pleural fluid may be present due to tuberculosis, viral infections, connective tissue disorders, Hodgkin's lymphoma and secondary to cardiac surgery [16]. Cytological examination is the most commonly used method; however, it might be very difficult to recognise and distinguish abnormal plasma cells in pleural fluid due to varying morphology, particularly, in the pauci-cellular samples. In addition, degeneration and sample processing cause changes in the morphology of the cells posing further diagnostic challenge. In such cases where cytology gives equivocal results flow cytometry may be used to supplement the diagnosis [5]. Flow cytometry immunophenotyping can separate these reactive cells from the malignant due their to aberrant antigen expression.

The initial specific identification or gating of plasma cells is best done by a combination of CD138, CD38, CD45 and light scatter characteristics or at least two markers, preferably CD38 and CD138 combination [17]. The most commonly assessed antigens for the differentiation of neoplastic and normal plasma cells include CD19, CD56, CD20, CD117, CD28, CD27, CD81 and CD200. Other markers used include CD31, CD39, CD40, CD44, cyclin D1, and CD34 [17–19]. Normal plasma cells usually express CD19, CD38, CD138 and are negative for CD20 and CD56. Neoplastic plasma cells display aberrant phenotypes and restricted immunoglobulin light chain expression at the cytoplasmic and sometimes at the surface membrane level. The aberrant plasma cell phenotype includes underexpression of markers like CD19, CD38, CD45, CD27, CD81 and overexpression of CD56, CD20, CD117, CD28 and CD200 [17, 19, 20]. In our case also, the plasma cells had aberrant expression of CD56 and absence of CD19, indicating their neoplastic nature.

The combined assessment of the expression of CD38, CD19, CD56, and CD45 is reliable for the specific identification of monoclonal plasma cells in >90 % of patients [19]. In the remaining cases, additional staining for cytoplasmic light chain or other potentially aberrant markers (e.g. CD117, CD81, CD20, CD27) may be required [17, 20]. Therefore, the demonstration of restricted immunoglobulin

coupled with an abnormal immunophenotype can be used to distinguish between reactive and neoplastic conditions.

Another very simplified immunophenotypic panel that includes CD56/CD19/CD138(CD38)/CD45 to distinguish neoplastic from reactive plasma cells has been recently proposed by Jeong et al. [21].

Thus, a number of antigens can be used to identify neoplastic and normal plasma cells, but, no single marker can reliably differentiate neoplastic plasma cells from their normal counterparts [17].

Conclusion

Myelomatous pleural effusion is a rare complication of plasma cell myeloma. Flow cytometry can be used as an adjunctive technique in its diagnosis particularly in cases with equivocal cytology and electrophoresis findings.

Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest.

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