# CHAPTER 6

# Hypereosinophilia

In the peripheral blood, eosinophil granulocytes are normally present in a concentration of $0.05–0.5 \times 10^9$/l, equal to 0.2–5% of circulating leucocytes. Classical criteria identify hypereosinophilia (or simply eosinophilia) as mild, moderate or severe when the concentration of eosinophils is respectively greater than an absolute count of 0.5, 1.5 or $5.0 \times 10^9$/l.[1] The value of $1.5 \times 10^9$/l is also that established as a threshold for classification of neoplastic disorders with eosinophilia in the 2008 World Health Organization (WHO) classification.[2]

Table 6.1 shows the principal causes of hypereosinophilia subdivided into:
- Reactive;
- Clonal;
- Idiopathic.[3–10]

In considering a differential diagnosis it is important to underline certain fundamental concepts:
- There are no unequivocal morphological or flow cytometric criteria that distinguish between reactive, clonal and idiopathic hypereosinophilias;
- The search for a possible cause of reactive eosinophilia is usually preliminary to other diagnostic approaches and complements the diagnostic approach to eosinophilia associated with haematological neoplasia;
- The identification of molecular genetic lesions is fundamental in the diagnostic pathway in cases that are not obviously reactive;
- Tissue and organ damage resulting from eosinophil infiltration occur independently of the cause of the eosinophilia.

Reactive hypereosinophilia can be associated with pathological processes such as infections (particularly parasitic infections), allergies, autoimmune disorders and neoplasms (Figs 6.1 through to 6.4). Hypereosinophilia is regulated by haemopoietic growth factors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and various interleukins (IL-2, IL-3, IL-5), IL-5 regulating specifically the production of eosinophils. Reactive eosinophilia results from the release of these cytokines in diverse pathological conditions or their release by various pathological or neoplastic cells. In the presence of a haematological neoplasm with associated eosinophilia, it is important, although not always possible, to establish whether or not the eosinophils are part of the neoplastic clone. A reactive eosinophilia is often seen in lymphoid neoplasms, including T-lineage lymphoproliferative disorders such as Sézary syndrome and angioimmunoblastic T-cell lymphoma,[11] B- or T-lineage lymphoblastic leukaemia/lymphoma (see Fig. 8.5), Hodgkin lymphoma and multiple myeloma; it is less common in other B-lineage lymphomas. A particular form of reactive eosinophilia is that secondary to the presence of a T-lymphoid population, which may or may not be clonal, but which often has an anomalous phenotype and is producing cytokines, in particular IL-5: this form of hypereosinophilia was originally described as 'idiopathic',[12] but nowadays must be recognised as reactive.

Clonal eosinophilias are subdivided into two groups on the basis of the presence or absence of molecular rearrangement of the *PDGFRA*, *PDGFRB* or *FGFR1* genes, which encode tyrosine kinase receptors involved in eosinophil proliferation.[13] The first group comprises the neoplasms, either myeloid or myeloid–lymphoid, in which it is possible to demonstrate molecular alteration of *PDGFRA*, *PDGFRB* or *FGFR1*. The second group includes chronic eosinophilic leukaemia, not otherwise specified (CEL, NOS) of the 2008 WHO classification and all the other conditions – myeloproliferative, acute myeloid or acute biphenotypic leukaemia described in other chapters of this book, in which the eosinophils in the peripheral blood do not represent the principal alteration, but are an integral part of the clonal expansion.

The term 'idiopathic hypereosinophilia' is used to designate the clinicopathological picture when neither the diagnostic criteria for reactive hypereosinophilia nor those for clonal eosinophilia are met.[14,15] It is thus a diagnosis of exclusion based on the presence of an eosinophil count of at least $1.5 \times 10^9$/l without an increase of blast cells, persisting for more than six months, in subjects in whom all the causes of reactive eosinophilia (see Table 6.1) have been excluded and, similarly, all the possible clonal lesions described below have been excluded. Idiopathic hypereosinophilia that is not demonstrably clonal or reactive, and is associated with signs of tissue infiltration and

**Table 6.1** Principal causes of eosinophilia

**Reactive eosinophilia**

*Non-neoplastic conditions*

- Allergic and atopic disorders (asthma, allergic rhinitis, allergic pneumonitis, hard metal pneumoconiosis, urticaria, eczema, atopic dermatitis, milk protein allergy, cyclical oedema with eosinophilia, drug reactions)
- Parasitic infections (visceral larva migrans, trichinosis, ascariasis, strongyloidiasis, ankylostomiasis, tapeworm infestation, echinococcosis, filariasis and tropical pulmonary eosinophilia, schistosomiasis, amoebiasis, fasciolaiasis, *Pneumocystis jirovecii* infection, *Toxocara canis* infection, toxoplasmosis)
- Non-parasitic infections (aspergillosis, coccidioidomycosis, infectious mononucleosis, mycobacterial infection, scarlet fever, brucellosis, cat scratch disease, infective lymphocytosis)
- Respiratory disorders (Churg-Strauss syndrome, Loeffler syndrome, cystic fibrosis, bronchiectasis)
- Endocrine disorders (adrenal insufficiency)
- Vasculitis and connective tissue disorders (rheumatoid arthritis, polyarteritis nodosa, Wegener granulomatosis, eosinophilic fasciitis)
- Gastrointestinal disorders (allergic gastroenteritis, coeliac disease)
- Cutaneous disorders (atopic dermatitis, eczema, pemphigus)
- Familial eosinophilia, including hereditary immunodeficiency associated with increased IgE
- Rare syndromes associated with eosinophilia such as Omenn syndrome
- Syndrome of L-tryptophane exposure
- Other causes (cirrhosis, radiotherapy, peritoneal dialysis)

*Paraneoplastic conditions*

- Carcinoma or sarcoma (lung, pancreas, colon, cervix, ovary)
- Hodgkin lymphoma
- T-cell leukaemia/lymphoma, including that due to the presence of an occult T-cell clone
- B-cell acute leukaemia/lymphoblastic lymphoma with t(5;14)(q31;q32)
- Langerhans cell histiocytosis
- Multiple myeloma and other haematological neoplasms (release of eosinophilopoietic cytokines by tumour cells)

**Clonal eosinophilia**

- Chronic eosinophilic leukaemia, not otherwise specified
- Haematological neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*
- Chronic myeloid leukaemia *BCR-ABL1*-positive with eosinophilia
- Acute myelomonocytic leukaemia with inv(16) or t(16;16) and eosinophilia
- Acute myeloid leukaemia with t(8;21) or not otherwise specified with (eosinophilic) maturation
- Myeloproliferative neoplasm, *JAK2* V617F-positive, with eosinophilia
- Aggressive systemic mastocytosis with eosinophilia
- Indolent systemic mastocytosis with eosinophilia
- Myelodysplastic syndrome with eosinophilia
- Myelodysplastic/myeloproliferative neoplasm with eosinophilia

**Idiopathic eosinophilia**

- Idiopathic hypereosinophilia (without organ damage)
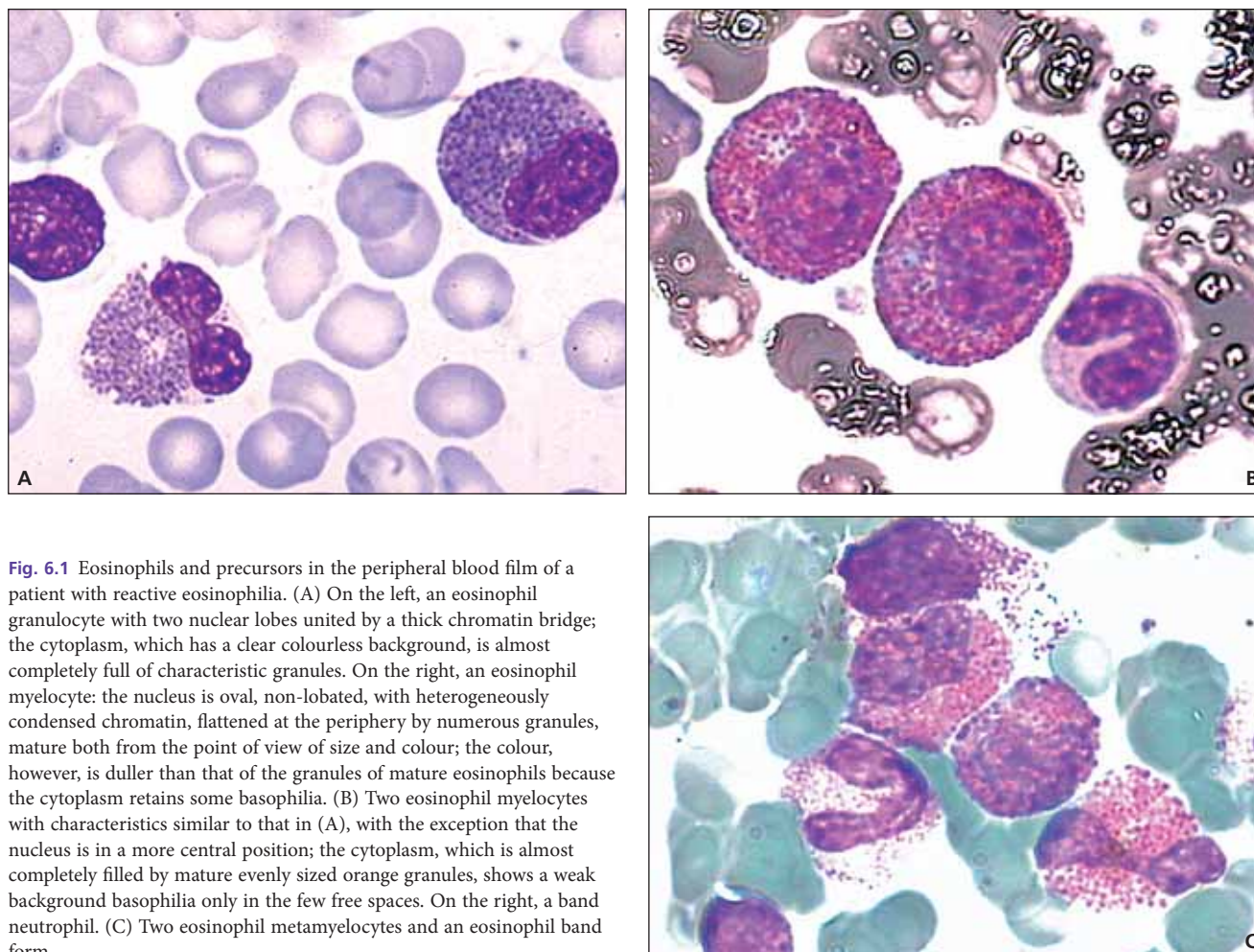- Idiopathic hypereosinophilic syndrome (with organ damage)







**Fig. 6.1** Eosinophils and precursors in the peripheral blood film of a patient with reactive eosinophilia. (A) On the left, an eosinophil granulocyte with two nuclear lobes united by a thick chromatin bridge; the cytoplasm, which has a clear colourless background, is almost completely full of characteristic granules. On the right, an eosinophil myelocyte: the nucleus is oval, non-lobated, with heterogeneously condensed chromatin, flattened at the periphery by numerous granules, mature both from the point of view of size and colour; the colour, however, is duller than that of the granules of mature eosinophils because the cytoplasm retains some basophilia. (B) Two eosinophil myelocytes with characteristics similar to that in (A), with the exception that the nucleus is in a more central position; the cytoplasm, which is almost completely filled by mature evenly sized orange granules, shows a weak background basophilia only in the few free spaces. On the right, a band neutrophil. (C) Two eosinophil metamyelocytes and an eosinophil band form.
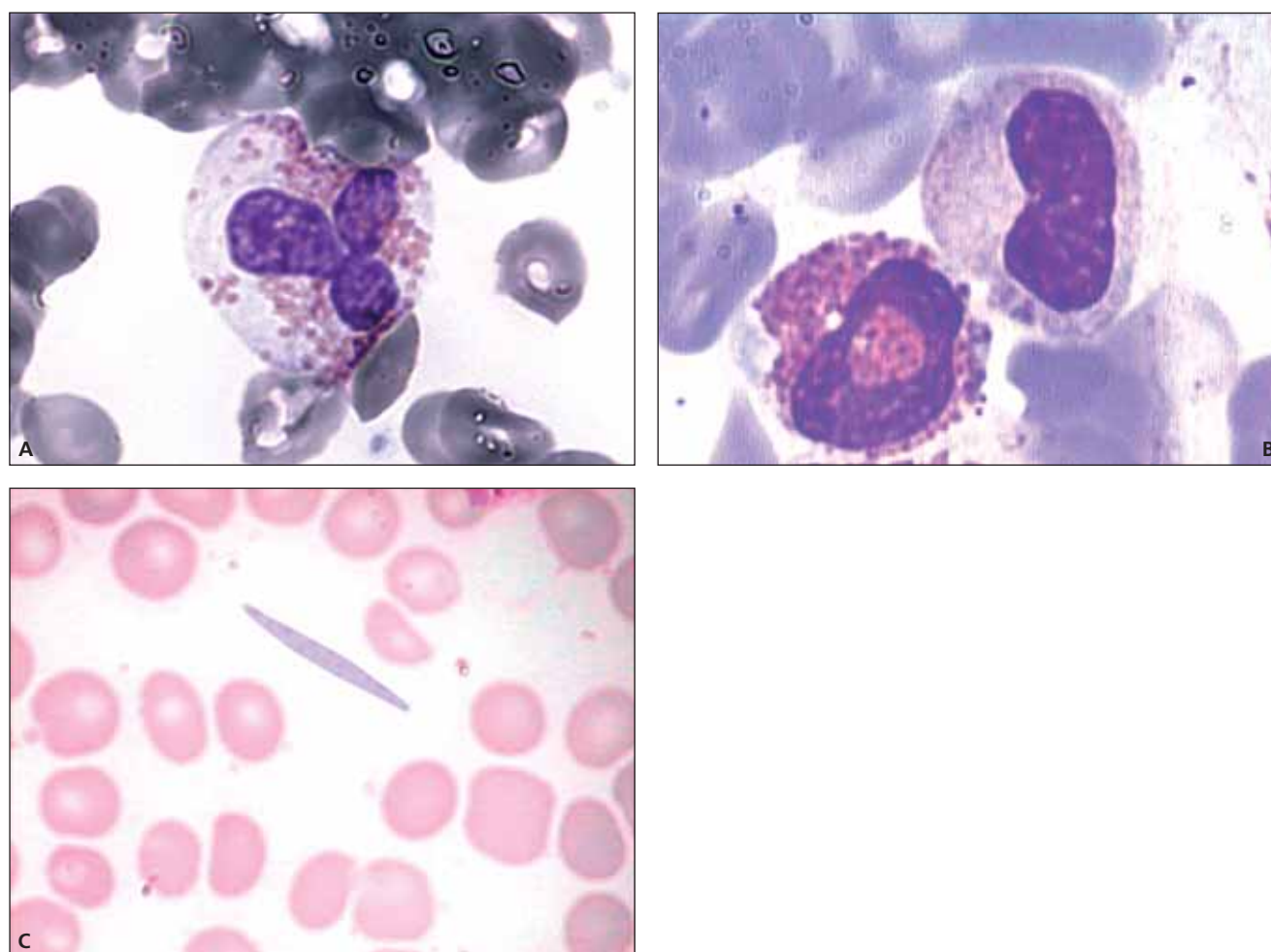
**Fig. 6.2** Dismorphism of mature eosinophils in patients with eosinophilia of various origins; peripheral blood film. Similar abnormalities are seen in reactive eosinophilia, but with a lower frequency than in clonal eosinophilia, associated with myelodysplasia (see also Figs 3.18 and 4.71) or with specific molecular rearrangements. (A) Eosinophil with a trilobed nucleus, in the form of a clover leaf; one of the lobes is twice the size of the other two. The granules appear mature but are scanty and irregularly distributed in cytoplasm that is grey with a bluish tinge. From the same blood film as Fig. 7.9. (B) Eosinophil with a ring nucleus in the peripheral blood of a patient with reactive eosinophilia. (C) Charcot-Leyden crystal in the bone marrow aspirate of a patient with a hypereosinophilic syndrome.
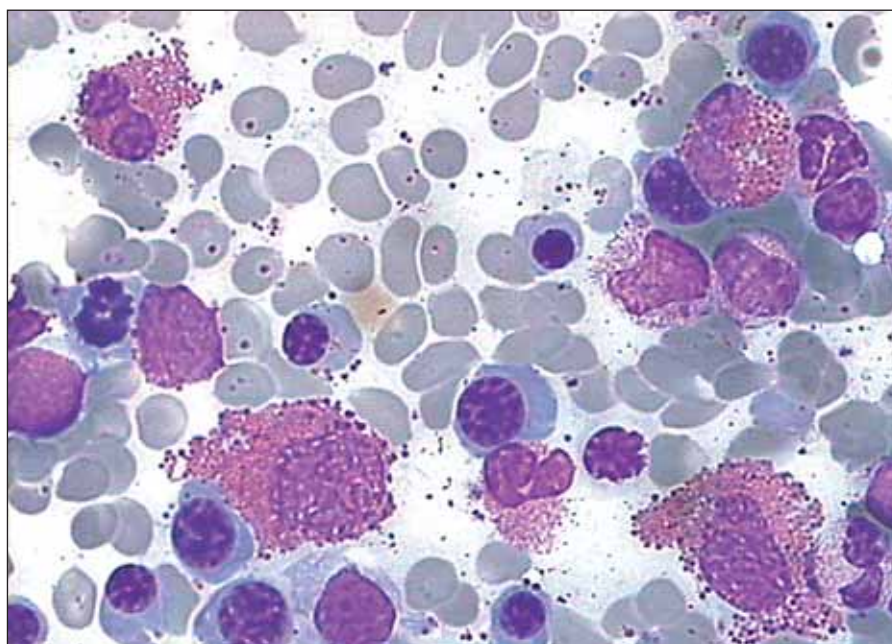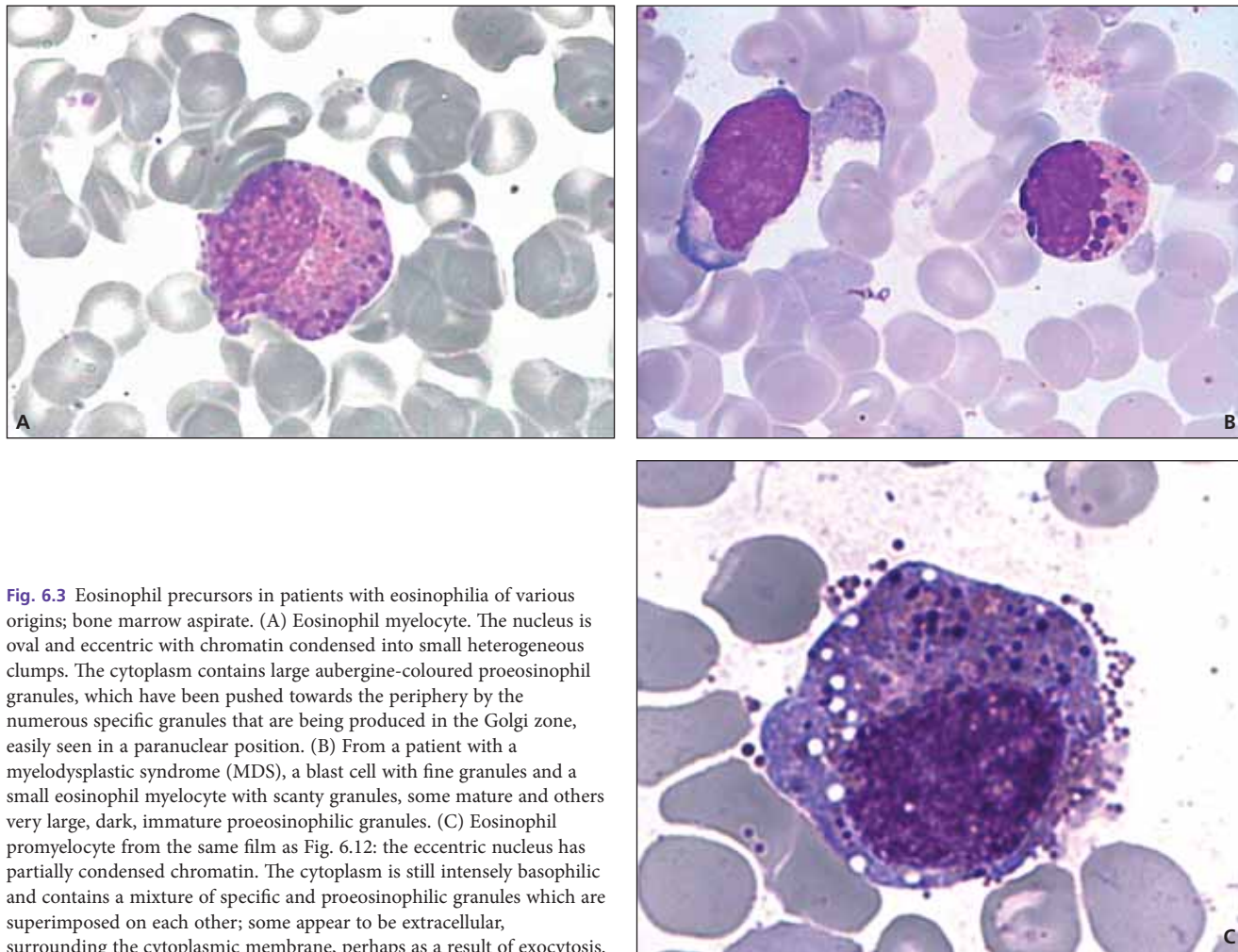
organ damage similar to those described for CEL, NOS, is designated the 'idiopathic hypereosinophilic syndrome' (Fig. 6.5, Report 6.1). It is possible that this group may include both some cases of eosinophilic leukaemia in which it is not currently possible to recognise the presence of clonal alterations, and some cases of reactive eosinophilia, secondary to release of cytokines as a result of a non-identifiable underlying condition.

The incidence of myeloproliferative hypereosinophilia in a study that included both clonal and 'essential' cases was 0.036 per 100 000 individuals per year, with a male predominance (M : F ratio of 1.47) and a peak incidence between 65 and 74 years, although some cases were observed in a younger group. Around 20% of patients with a diagnosis of 'hypereosinophilic

syndrome' have rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*.[16]

## Chronic eosinophilic leukaemia, not otherwise specified

This is a rare haematological neoplasm included under this designation in the MPN group in the 2008 WHO classification, characterised by a persistent clonal proliferation of progenitors showing eosinophilic differentiation: as a consequence there is an expansion of the eosinophil component, often with well-preserved maturation, in the bone marrow, in the peripheral
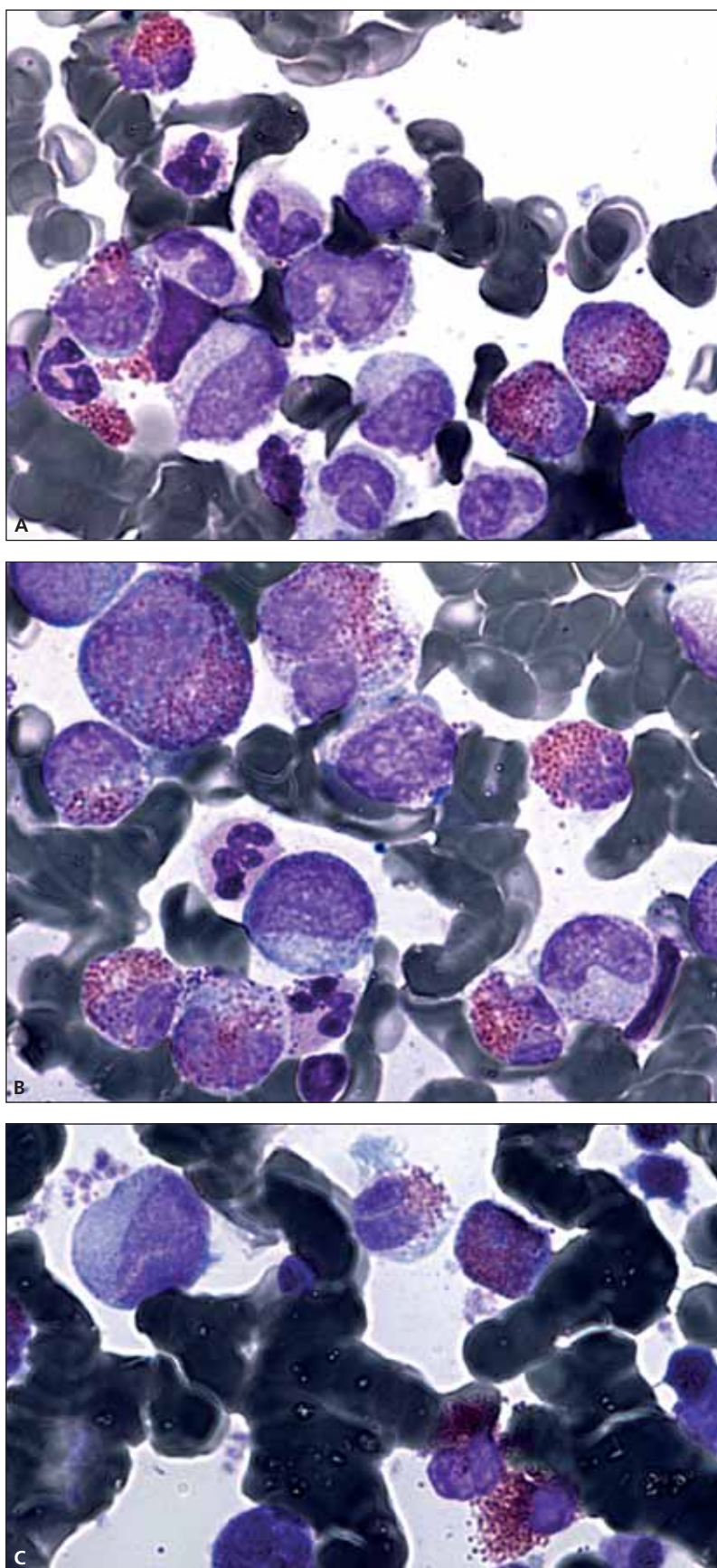
**Fig. 6.3** Eosinophil precursors in patients with eosinophilia of various origins; bone marrow aspirate. (A) Eosinophil myelocyte. The nucleus is oval and eccentric with chromatin condensed into small heterogeneous clumps. The cytoplasm contains large aubergine-coloured proeosinophil granules, which have been pushed towards the periphery by the numerous specific granules that are being produced in the Golgi zone, easily seen in a paranuclear position. (B) From a patient with a myelodysplastic syndrome (MDS), a blast cell with fine granules and a small eosinophil myelocyte with scanty granules, some mature and others very large, dark, immature proeosinophilic granules. (C) Eosinophil promyelocyte from the same film as Fig. 6.12: the eccentric nucleus has partially condensed chromatin. The cytoplasm is still intensely basophilic and contains a mixture of specific and proeosinophilic granules which are superimposed on each other; some appear to be extracellular, surrounding the cytoplasmic membrane, perhaps as a result of exocytosis.





**Fig. 6.4** Reactive hypereosinophilia; bone marrow aspirate. There are numerous polychromatophilic and orthochromatic erythroblasts with retardation of nuclear maturation and a mitotic figure on the left; top right, two small metamyelocytes, a neutrophil granulocyte, a lymphocyte and a large mature eosinophil with a bilobed nucleus. At the bottom, two very spread out eosinophil myelocytes with a partially damaged cytoplasmic membrane; there is another mature eosinophil top left.

**Fig. 6.5** Idiopathic hypereosinophilic syndrome; bone marrow aspirate. (A) There are various neutrophil and eosinophil granulocytes at different stages of maturation. (B) Top left, a late promyelocyte with basophilic cytoplasm, loaded with specific granules; immediately beneath there is a small eosinophil metamyelocyte. At the top, a large bilobed eosinophil, incompletely granulated; another similar cell is found near to the lower edge of the image. The other mature eosinophils have a more normal appearance. (C) Mature dysmorphic eosinophils from the same film. The upper one has two elongated nuclear lobes like a saddlebag and scanty granules concentrated in a paranuclear position, leaving free large areas of blue cytoplasm. At the bottom, an eosinophil with a non-lobed nucleus.

blood and in tissues.[17] The pathological effects are systemic since degranulation of eosinophils leads to release of cytotoxic proteins, cytokines and humoral factors that cause damage to numerous organs and systems. Among these effects, there can be fibrosis and cardiac valvular lesions, cough resulting from bronchospasm, pulmonary fibrosis, involvement of the central nervous system, peripheral neuropathy, cutaneous lesions such as angio-oedema, urticaria and erythema, venous and arterial thrombosis, diarrhoea, joint disorders of a rheumatic type and ocular alterations.[18,19] In a manner analogous to other MPN, around a half of patients present with hepatosplenomegaly.

The WHO 2008 diagnostic criteria for CEL, NOS are met when there are[2]:

- Peripheral blood eosinophils of at least $1.5 \times 10^9$/l;
- Absence of the Philadelphia chromosome, *BCR-ABL1* fusion or both;
- Absence of the criteria required for a diagnosis of other MPN: polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF);
- Absence of the criteria required for a diagnosis of other myelodysplastic/myeloproliferative neoplasms (MDS/MPN): chronic myelomonocytic leukaemia (CMML) and atypical chronic myeloid leukaemia (aCML);
- Peripheral blood and bone marrow blast cells less than 20% (to exclude a diagnosis of acute myeloid leukaemia [AML]);
- Absence of inv(16)(p13q22) and t(16;16)(p13;q22) and of other alterations diagnostic of AML;
- Absence of *FIPL1-PDGFRA* and other rearrangements of *PDGFRA*;
- Absence of t(5;12)(q31–35;p13) and other rearrangements of *PDGFRB*;
- Absence of rearrangement of *FGFR1*;
- Recognition of a clonal cytogenetic or molecular genetic abnormality (other than those cited above) or an increase of blast cells, equal to or greater than 2% in the blood or 5% in the bone marrow, or evident dysplasia in one or more haemopoietic lineages;
- Exclusion of all causes or reactive eosinophilia, including paraneoplastic eosinophilia.

In summary, the diagnosis of CEL, NOS is made, on the one hand, by exclusion of the above-mentioned specific genetic lesions and other conditions and, on the other hand, by the demonstration of clonality or evidence of myeloid neoplasia furnished by an increase of blast cells or the association with severe dysplastic changes in other lineages. No molecular alterations have been identified that are pathognomonic for CEL, NOS: the cytogenetic abnormalities most often observed are non-specific, such as trisomy 8 or isochromosome 17p. The presence of clonality can sometimes be demonstrated in females by analysis of polymorphism in X chromosome genes such as *PGK2* or *AR* (HUMARA).[20]

With regard to morphology, in the peripheral blood one observes mainly mature eosinophils, with a variable percentage, not usually very high, of eosinophil metamyelocytes, myelo-cytes and promyelocytes. The eosinophils can be morphologically normal or they can be altered in size or show various cytomorphological abnormalities; the latter vary in degree and can include reduced segmentation (pseudo-Pelger type), increased segmentation and bizarre shapes such as ring nuclei.[21] The cytoplasm may show the presence of vacuoles, hypogranularity, irregular distribution of granules with clear or weakly basophilic agranular areas, reduced granule size or granule fusion to form small inclusions that resemble miniature Charcot-Leyden crystals. The precursors can have prominent violaceous proeosinophilic granules. All these types of eosinophil dysmorphism are non-specific and therefore cannot be used to assign a particular case to the clonal, reactive or idiopathic categories. The morphological observations that are useful for excluding a reactive or idiopathic condition are the recognition at the time of diagnosis of increased blast cells, either granular or agranular, in the peripheral blood or bone marrow or the recognition of dysplasia in other lineages. In addition to eosinophilia, neutrophilia is common; less frequent is monocytosis or basophilia. Anaemia and thrombocytopenia are seen in the more advanced stages of the disease.

The bone marrow is hypercellular with a marked increase in the percentage of eosinophils, generally with preservation of normal maturation. There can be dysmorphism of immature eosinophils (Fig. 6.6). The percentage of blast cells may be increased, but by definition is less than 20%. One may observe, as in other types of hypereosinophilia, Charcot-Leyden crystals of a typical elongated rhomboidal or diamond shape, like double pyramids joined by their bases, sometimes orange or rose in colour, which are often found in the expectorate or nasal secretions of allergic subjects and in the faeces of patients with parasitic infections; they are formed from phospholipase liberated from eosinophil granules.[22,23] Other bone marrow lineages are usually normal. An increase in myeloblasts and the recognition of dysgranulopoiesis, severe dyserythropoiesis or dysmegakaryopoiesis exclude the diagnosis of reactive eosinophilia and these are therefore important features that sustain a diagnosis of CEL. There is almost always reticulin fibrosis as a result of eosinophil degranulation.[24]

From the immunophenotypic point of view, clonal eosinophils do not show any specific markers.

## Hypereosinophilia associated with clonal myeloid disorders

In this form of hypereosinophilia, the eosinophils are almost always part of the neoplastic clone. Only in rare cases has a reactive nature been shown.[4] The neoplastic disorders that are most often characterised by involvement of the eosinophil lineage with hypereosinophilia are:

- AML with recurrent cytogenetic abnormalities (particularly acute myelomonocytic leukaemia with chromosome 16 aberrations; see Figs 4.25 and 4.31);
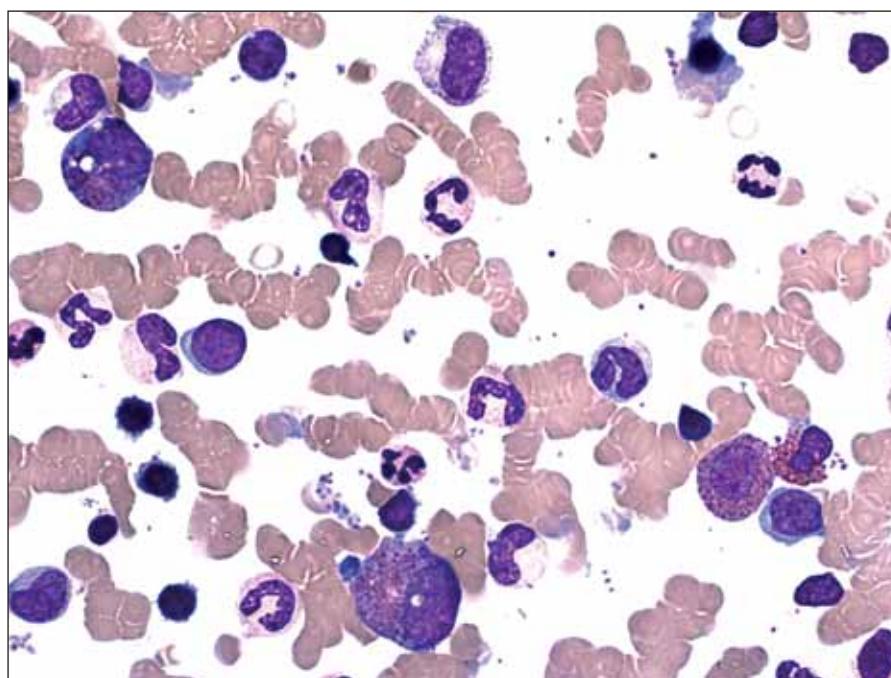
**Fig. 6.6** Infiltration by eosinophils at all stages of maturation in another patient with idiopathic hypereosinophilic syndrome; bone marrow aspirate. In the top left corner and in the centre at the bottom, two eosinophil promyelocytes; in the middle between them is a small agranular blast cell. On the right, an eosinophil myelocyte and an eosinophil promyelocyte. The red cells are forming long rouleaux.

- MPN, particularly *BCR-ABL1*-positive CML and systemic mastocytosis[25,26];
- MDS/MPN (CMML, juvenile myelomonocytic leukaemia [JMML] and aCML);
- Some MDS (see Fig. 3.18).

## Myeloid and lymphoid neoplasms with alteration of *PDGFRA, PDGFRB* or *FGFR1*

The neoplasms associated with rearrangement of one of the three genes that encode platelet-derived growth factors A (*PDGFRA*) or B (*PDGFRB*) or the receptor for fibroblast growth factor 1 (*FGFR1*) have variable characteristics, involving both myeloid and lymphoid cells; they thus have quite variable clinicopathological presentations, including AML, MPN, MDS/MPN and lymphoid neoplasms.

These disorders are dealt with in a specific chapter of the 2008 WHO classification.[27] The pathogenetic element that unites them is a molecular alteration that leads to the encoding of one of three different tyrosine kinases implicated in the neoplastic transformation. A fusion gene involving one of these three genes is created, each encoding a specific aberrant tyrosine kinase, which is activated and conveys a proliferative advantage and resistance to apoptosis; this favours the generation and expansion of an aberrant clone. Numerous partner genes involved in the generation of fusion genes have been described: the most frequent are *FIP1L1* as a partner of *PDGFRA*, *ETV6* (*TEL*) as a partner of *PDGFRB* and *ZNF198* as a partner of *FGFR1*.[28,29] The clinical picture and the course of the disease differ according to the type of rearrangement.

The recognition of rearrangement of *PDGFRA* or *FGFR1* in both myeloid and lymphoid neoplasms indicates derivation of these neoplasms from a mutated pluripotent stem cell. Further confirmation of the heterogeneity of the disorders included in this category is provided by their different responses to treatment with tyrosine kinase inhibitors, which are very efficacious when there is rearrangement of *PDGFRA* or *PDGFRB*, but not when there is a molecular alteration in *FGFR1*.[30]

### Neoplasms with rearrangement of *PDGFRA*

These conditions, in general, have very similar clinical and haematological features to CEL, NOS, with peripheral blood and bone marrow eosinophilia and the clinical picture of organ damage. They are much more common in men (M : F ratio of 17 : 1). Often there is a tendency to acute transformation. It is also possible for the initial presentation to be as AML, T-lineage lymphoblastic lymphoma or the simultaneous presentation of CEL and acute leukaemia or lymphoblastic lymphoma. According to the 2008 WHO classification, the presence in these neoplasms of rearrangement of *PDGFRA* is sufficient for inclusion of a case in this category.

The common feature is the presence of an often marked peripheral blood eosinophilia (at least $1.5 \times 10^9$/l). The eosinophils are mainly mature, but there can also be some eosinophil precursors at different stages of maturation (Figs 6.7 through to 6.10). As in CEL, NOS, the eosinophils can appear normal or can show cytoplasmic atypia (such as vacuolisation,
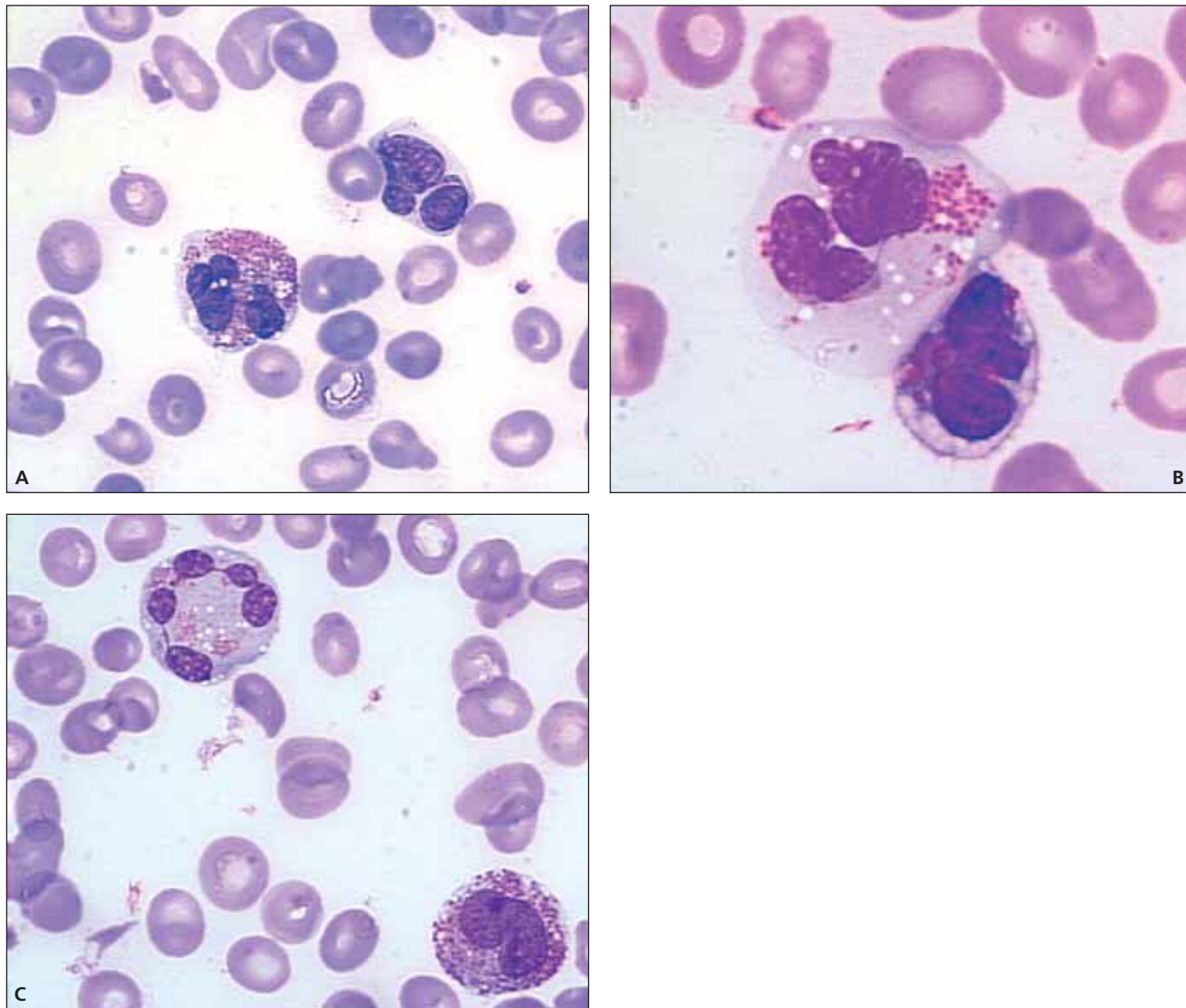
**Fig. 6.7** Myeloproliferative neoplasm (MPN) with hypereosinophilia and rearrangement of *PDGFRA*; peripheral blood film. The eosinophil series in this patient shows very striking dysmorphism. (A) On the left, an eosinophil granulocyte with an irregularly multilobated nucleus; the specific granules are small and numerous, but do not occupy all the cytoplasm, which is grey and contains some vacuoles. The cell on the right is certainly an eosinophil with similar nucleus and cytoplasm, but is completely agranular. (B) Two eosinophils, one giant and one contracted with an extreme grade of nuclear and cytoplasmic dysmorphism. For cells of this type, one can also use the designation 'dysplastic', which is generally not attributed to morphological abnormalities of eosinophils because of their lack of specificity. (C) Top left, a large eosinophil with a five-lobed nucleus, stretched out and almost forming a circle; the grey vacuolated cytoplasm contains a few clumps of fine specific granules. Bottom right, an eosinophil metamyelocyte, this also having very small granules.
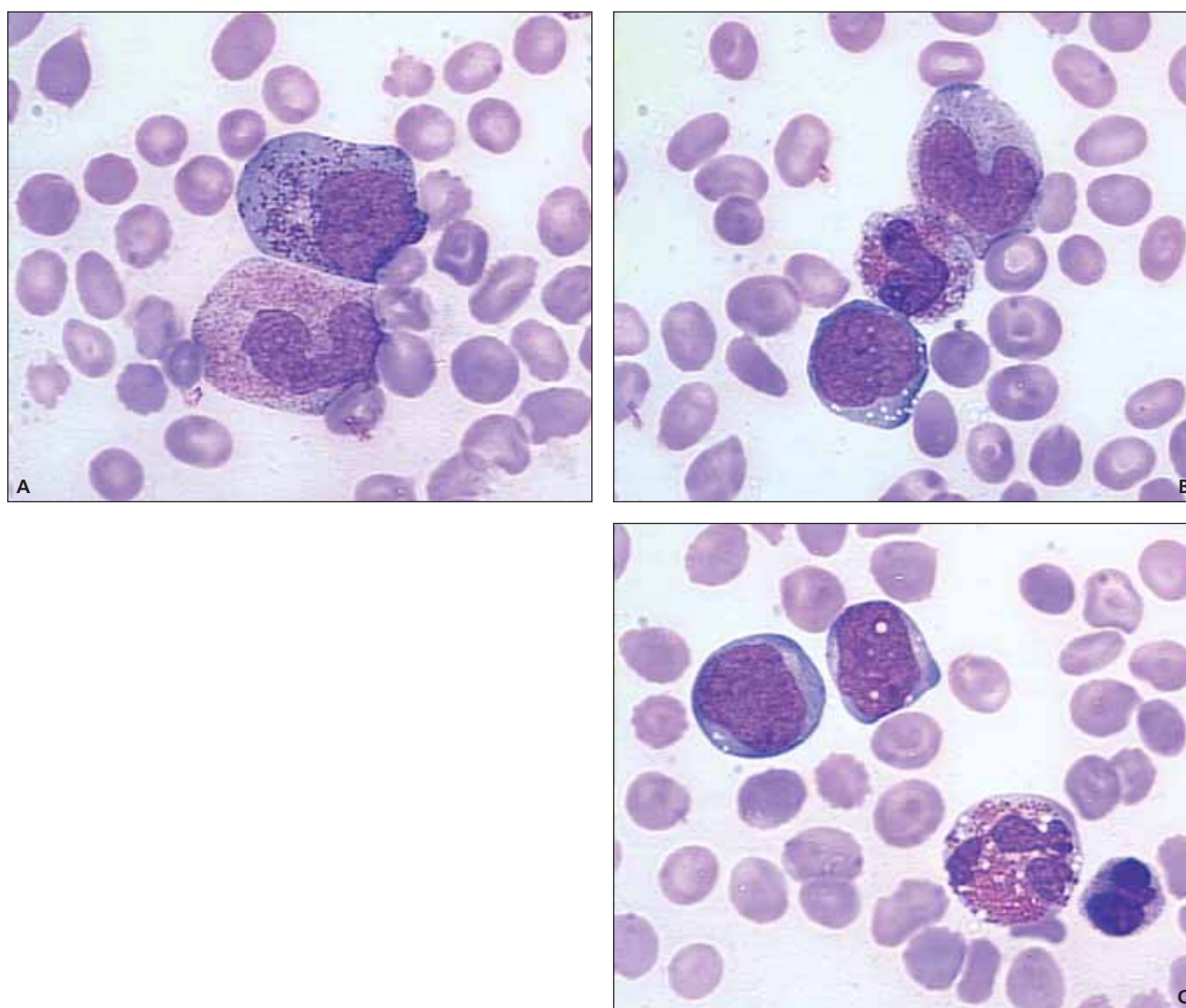
**Fig. 6.8** MPN with hypereosinophilia and rearrangement of *PDGFRA*; acute evolution in the patient shown in Fig. 6.7; peripheral blood film. (A) At the bottom, a dysplastic metamyelocyte with very fine granules dispersed in ample cytoplasm; the nucleus has immature chromatin. At the top, a dysplastic promyelocyte with ample basophilic cytoplasm and granules spread unevenly around an agranular paranuclear zone. The chromatin is reticular with some incompletely defined nucleoli. The possible eosinophil lineage of this cell is a supposition based on the general cytomorphological context of hypereosinophilia with severe dysmorphism. (B) At the top, an immature precursor with a horseshoe-shaped nucleus and reticular chromatin with nucleoli; it is not morphologically definable (granular blast cell or dysplastic promyelocyte?). At the bottom, an agranular blast cell with very fine homogeneous chromatin and two nucleoli. Between the two, an eosinophil with dysmorphism of the nucleus and granules that appear fine and dark. (C) On the left, two agranular blast cells with an angulated nuclear profile. On the right, an eosinophil with a four-lobed nucleus and an unrecognisable dwarf granulocyte.
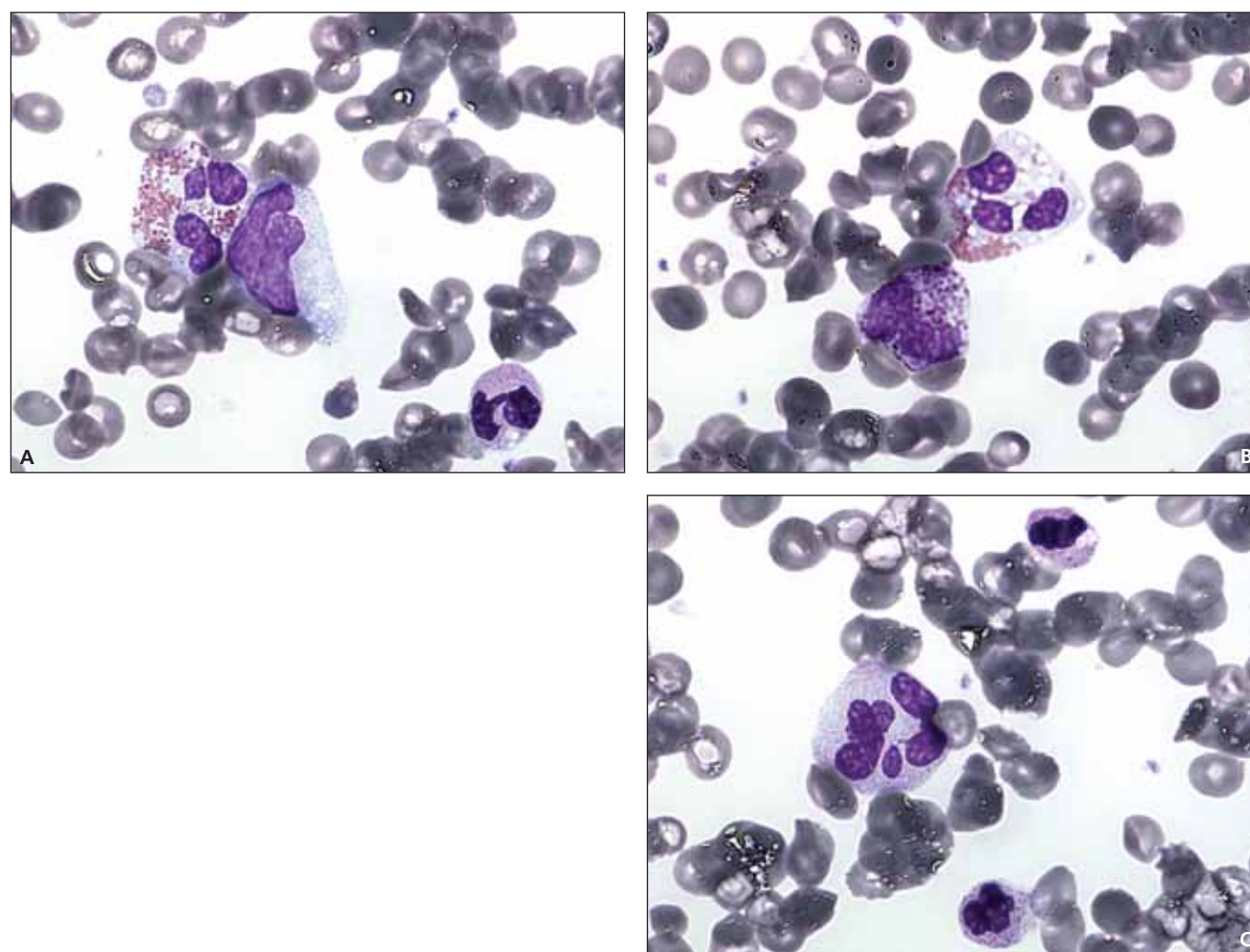
**Fig. 6.9** MPN with hypereosinophilia, basophilia, dysgranulopoiesis and rearrangement of *PDGFRA*; peripheral blood film. (A) On the left an eosinophil that has a multilobed nucleus and cytoplasm incompletely filled with granules; an atypical monocyte; a small neutrophil with very dense chromatin and abnormal segmentation. (B) A trilobed eosinophil with vacuolated cytoplasm and scanty granules almost all of which are clumped together in a limited area of the cytoplasm beneath the cytoplasmic membrane. At the bottom, an eosinophil metamyelocyte with proeosinophilic granules. (C) In the centre, a large dysplastic neutrophil granulocyte with pale hypogranular cytoplasm and a hyperlobated nucleus. At the top and at the bottom, another two dysplastic neutrophils, quite a deal smaller with almost pyknotic chromatin.

disorderly distribution of granules and immature purple granules), nuclear atypia or alterations in cell size. As already indicated, morphological alterations in eosinophils are non-specific and are found similarly in reactive and clonal eosinophilias. Sometimes at presentation there is anaemia, thrombocytopenia, neutrophilia or the presence of a small number of blast cells. Blast cells are more numerous when there is associated AML.

The bone marrow is usually hyperplastic with expansion of the eosinophil compartment, generally with retention of the capacity to mature (Figs 6.11 and 6.12). The percentage of blast cells is higher in cases with associated AML. There is often a marked increase in mast cells, so that the differential diagnosis includes systemic mastocytosis; the biological link between

eosinophils and mast cells, as seen in allergic conditions and Hodgkin lymphoma, is evident.[26] In histological sections, the mast cells are usually dispersed, but they can form cohesive aggregates with fusiform cells, making the bone marrow picture sometimes indistinguishable from that of systemic mastocytosis.[31] Charcot-Leyden crystals may be observed both in the bone marrow aspirate and in histological sections.[32] Areas of necrosis, increased reticulin and an increased number of blast cells are characteristic of the disease in evolution.

Laboratory investigations show an increase in serum vitamin $B_{12}$ and, often, an increase in serum tryptase. Immunohistochemical investigation is sometimes useful to show the mast cells' immunophenotype, these cells often being positive for
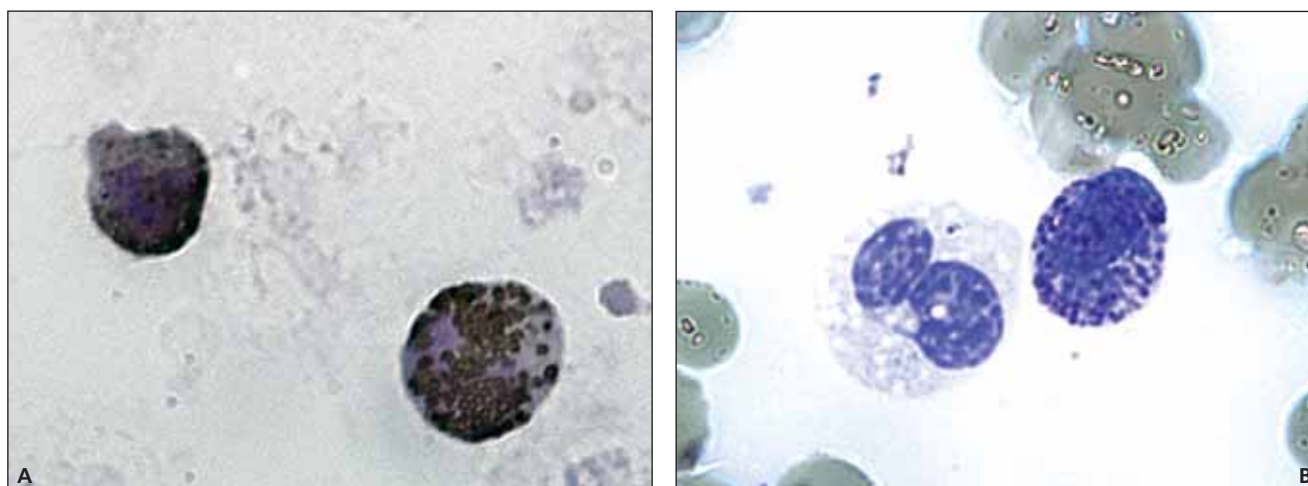
**Fig. 6.10** Myeloproliferative neoplasm with hypereosinophilia, basophilia, dysgranulopoiesis and rearrangement of *PDGFRA*; same case as Fig. 6.9, peripheral blood film. (A) Peroxidase reaction. The cell on the right shows the typical large granular positivity of the eosinophil lineage. (B) Toluidine blue. The granular metachromatic staining with a purplish tinge shown by the granulocyte on the right confirms basophil lineage. The granulocyte on the left has no positively-stained granules.

CD25 but, in contrast with the majority of cases of systemic mastocytosis, negative for CD2. Diagnosis requires the identification of the fusion gene by reverse transcription-polymerase chain reaction (RT-PCR) or fluorescence *in situ* hybridisation (FISH). In a minority of cases cytogenetic analysis shows a translocation such as t(1;4) or t(4;10) with a 4q22 breakpoint; in the majority of cases, however, the molecular lesion is cryptic and cytogenetic analysis is normal.

often associated with monocytosis: the percentage of blast cells, in the absence of acute evolution, must be, by definition, less than 20%. There may be an increase in mast cells and these may be fusiform.

The diagnosis is made with the aid of cytogenetic analysis and molecular analysis by RT-PCR. This disorder, like neoplasms with rearrangement of *PDGFRA*, responds well to treatment with tyrosine kinase inhibitors.

### Neoplasms with rearrangement of *PDGFRB*

In the presence of an *ETV6-PDGFRB* fusion gene resulting from t(5;12)(q31~33;p12) there is a characteristic picture of an MPN with eosinophilia; other molecular variants also resulting in *PDGFRB* rearrangement have been described. From the clinicopathological point of view, the features are most often those of a myelodysplastic/proliferative neoplasm (MDS/MPN) (see Chapter 7): the most frequently observed picture is that of CMML with eosinophilia, but cases have also been described with features very similar to aCML or CEL or, rarely, AML (Fig. 6.13). These cytomorphological variants are associated with hypereosinophilia in the majority of cases, but this is not an obligatory feature.[33] The peripheral blood typically shows leucocytosis, resulting from neutrophilia, eosinophilia and monocytosis, usually in the absence of basophilia or abnormal maturation. The bone marrow is hypercellular because of expanded, effective neutrophil and eosinophil granulopoiesis,

### Neoplasms with rearrangement of *FGFR1*

Rearrangement of *FGFR1* is associated with a very heterogeneous group of clinical disorders, suggesting that the mutation arises in a pluripotent haemopoietic stem cell. Depending on the lineage and the degree of differentiation of the expanding clone, these patients can present with an MPN, AML, T-lineage or B-lineage acute lymphoblastic leukaemia or an acute leukaemia of mixed phenotype. In the literature, patients with t(8;13) (p11;q12) and the *ZNF198-FGFR1* fusion gene have been designated as having the '8p11 myeloproliferative syndrome' or '8p11 stem cell syndrome'.

Eosinophilia has been recognised in more than 90% of the reported case, regardless of whether the clinicopathological presentation is as a chronic disorder, usually with associated neutrophilia and monocytosis, or as an acute leukaemia. Currently, the prognosis of these patients is grave; tyrosine kinase inhibitors are ineffective.
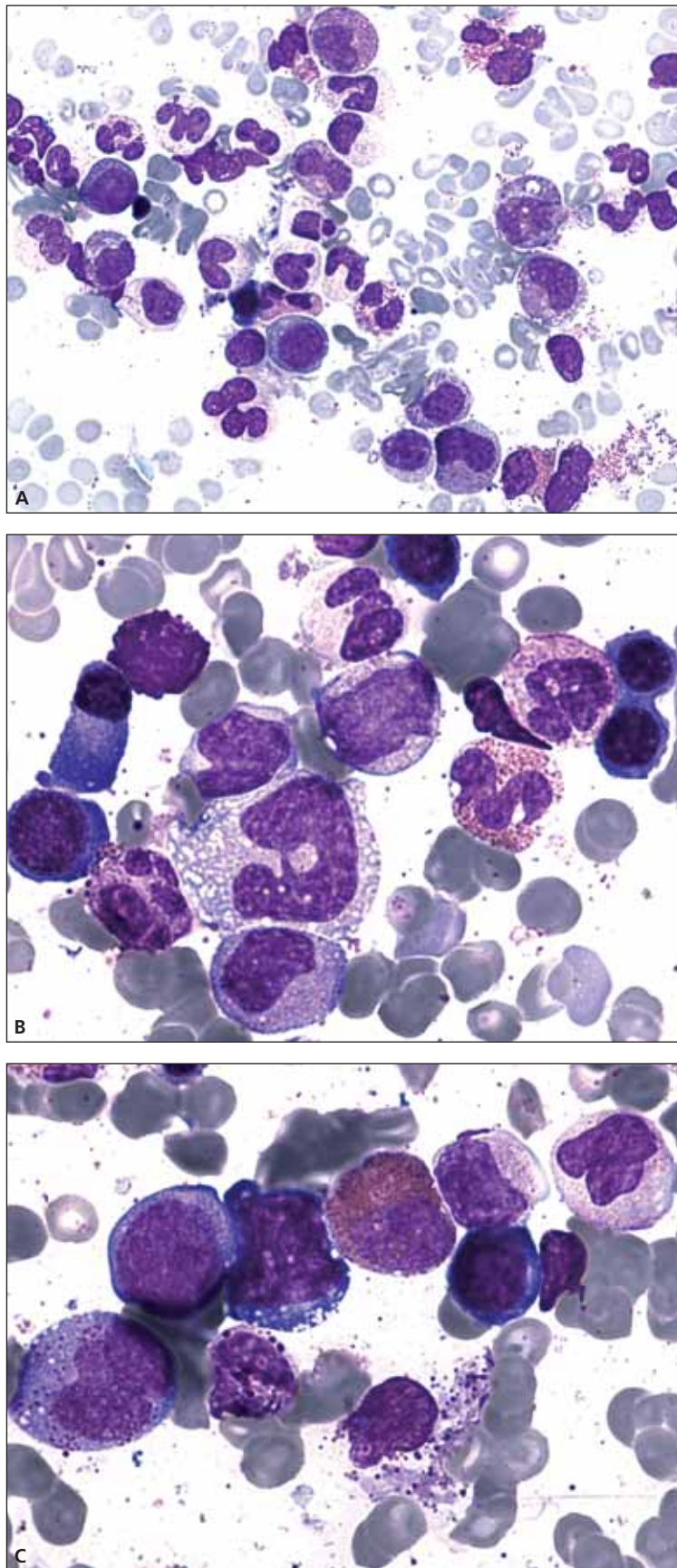
**Fig. 6.11** MPN with hypereosinophilia, basophilia, dysgranulopoiesis and rearrangement of *PDGFRA*; bone marrow aspirate of the same case as Figs 6.9 and 6.10. (A) The very cellular marrow is composed mainly of cells of neutrophil lineage at all stages of maturation: there are two promyelocytes (one just under the centre of the image, the other higher to the left) and various mature cells, in which dysplastic features are more evident. In the centre and on the right, several mature eosinophils, two of which have torn cytoplasm. (B) On the left, a basophil granulocyte, a small almost rectangular plasma cell and a basophilic erythroblast. In the central area, six dysplastic granulocytes in various stages of maturation. On the right, a mature neutrophil, an eosinophil band form and two polychromatic erythroblasts with cytoplasm not yet divided. (C) On the left, a dysplastic promyelocyte, a blast cell with fine granules, a large vacuolated and crushed basophilic cell (perhaps a proerythroblast), immediately beneath a small hypogranular basophil, an eosinophil metamyelocyte, a basophilic erythroblast and two dysplastic neutrophil metamyelocytes.
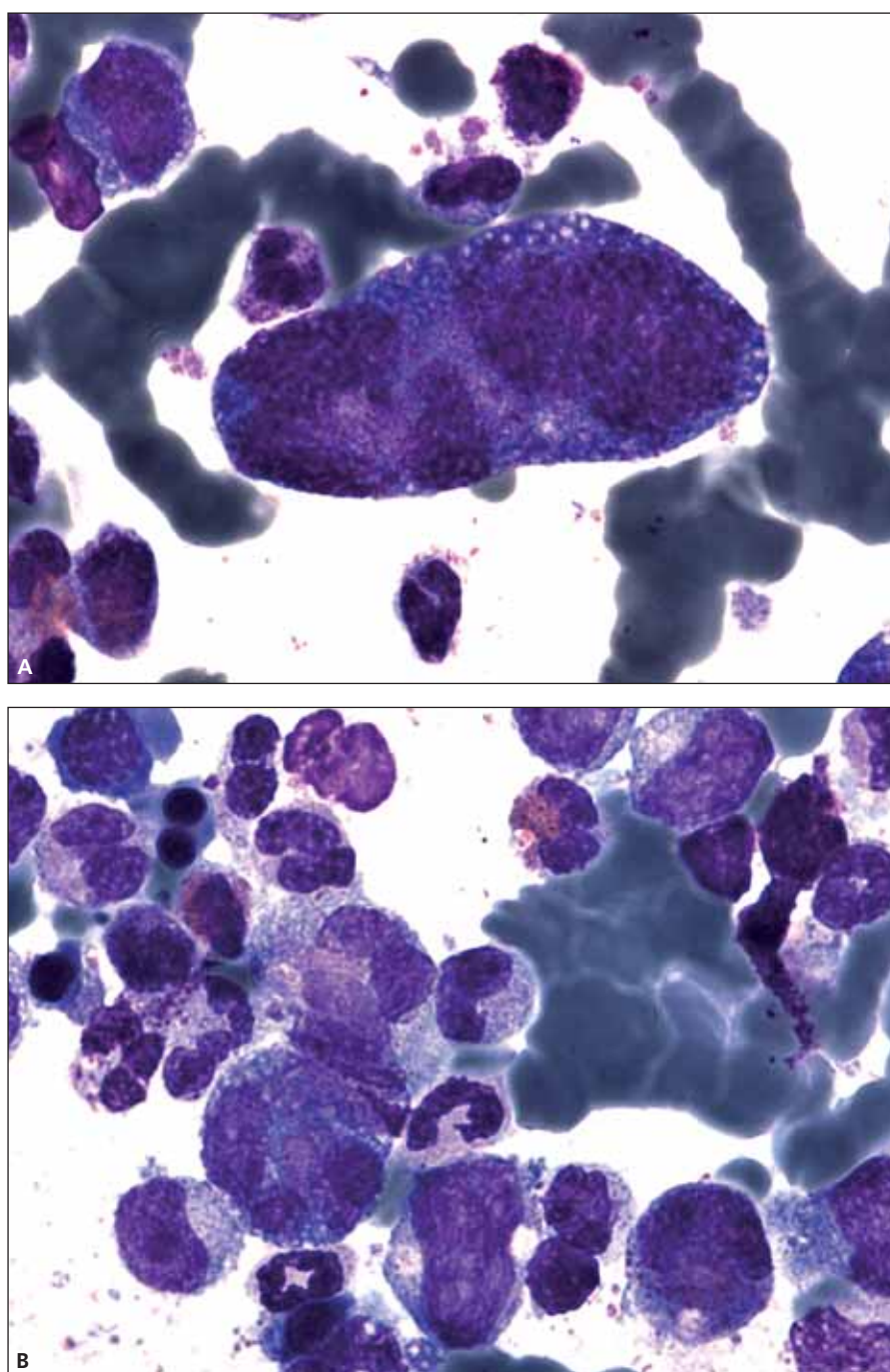
**Fig. 6.12** MPN with hypereosinophilia, basophilia, dysgranulopoiesis and rearrangement of *PDGFRA*; bone marrow aspirate, same case as Fig. 6.11. (A) A monstrous immature hyperdiploid and multinucleated granulocyte, with extensive intensely basophilic and granulated cytoplasm, certainly a dysplastic cell belonging to the neutrophil lineage. (B) Dysplastic neutrophil granulocyte, two eosinophils and two dysplastic erythroblasts. In the top right corner, a fusiform mast cell.
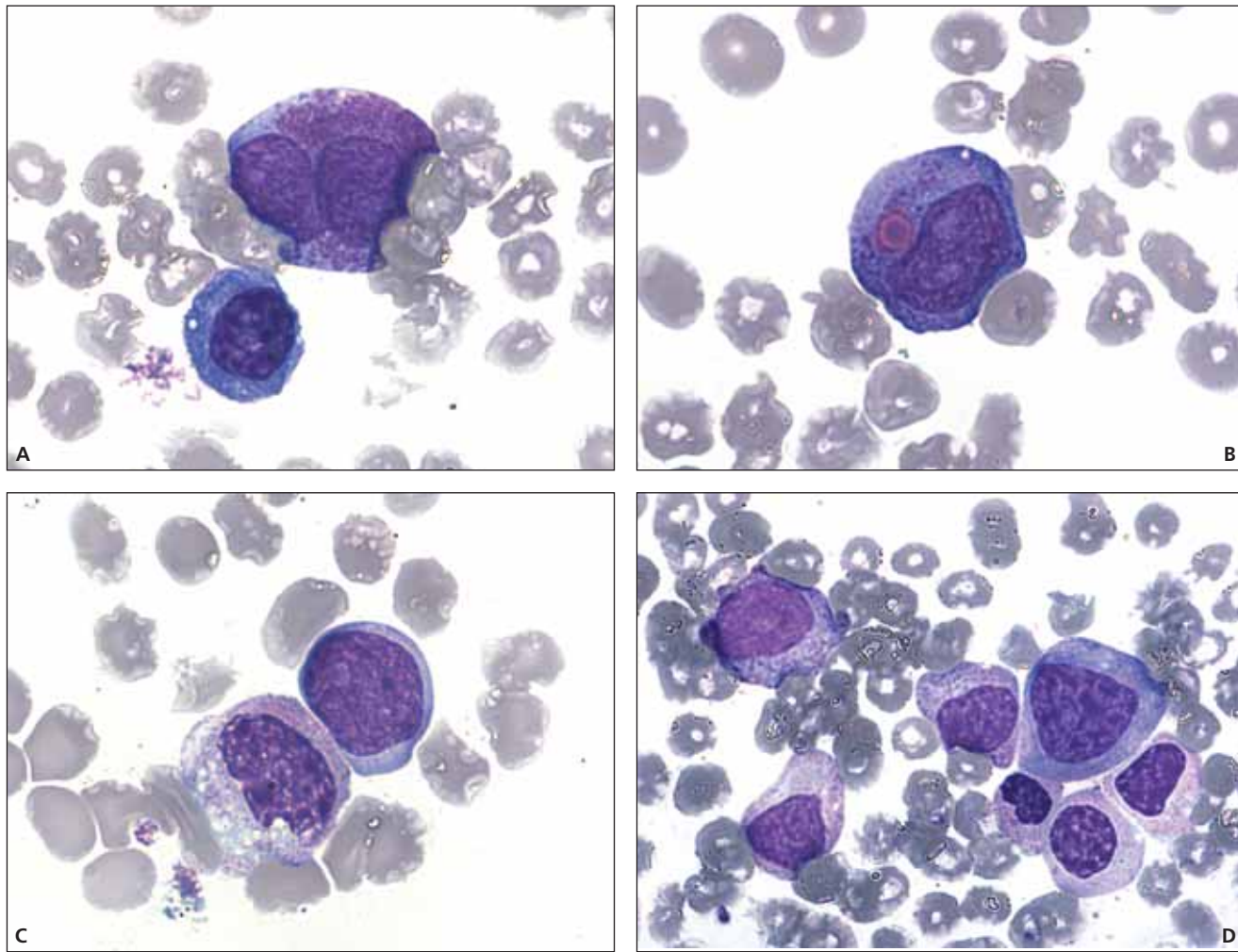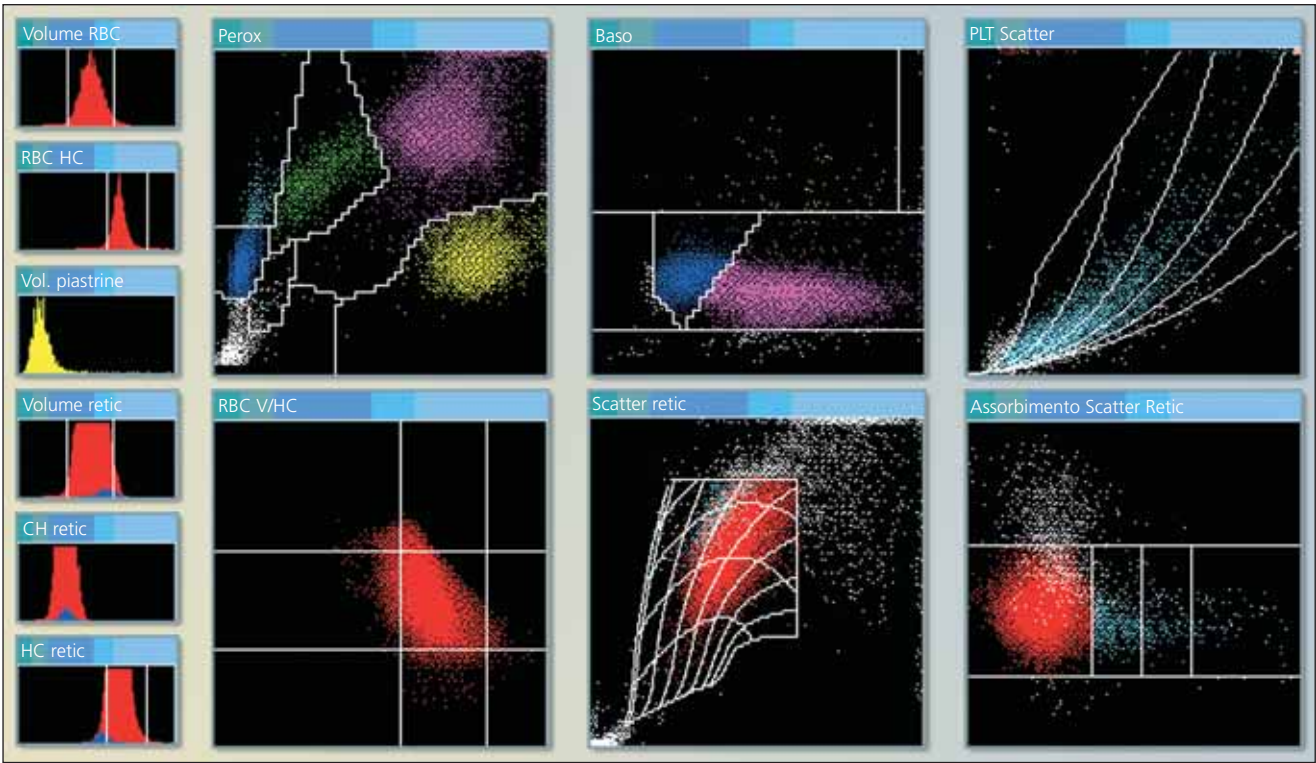
**Fig. 6.13** MDS/MPN with leucocytosis (WBC $14 \times 10^9$/l), eosinophilia and rearrangement of *PDGFRB* in a patient previously treated for carcinoma of the colon and subsequently small cell carcinoma of the lung (therapy-related myeloid neoplasm); peripheral blood film. (A) Large dysplastic eosinophil with immature chromatin; a dyserythropoietic basophilic erythroblast with nuclear chromatin in blocks. (B) Circulating dysplastic eosinophil promyelocyte with two nucleoli; the basophilic cytoplasm contains azurophilic granules and an enormous eosinophil granule of pseudo-Chédiak-Higashi type. (C) From the left: two dysmorphic platelets, a neutrophil myelocyte with vacuolated cytoplasm and an agranular blast cell. (D) Dysplasia of neutrophil granulocytes; top left, a dysplastic promyelocyte.

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 132 | g/l | |
| RBC | 4.47 | ×10$^{12}$/l | |
| HCT | 0.412 | l/l | |
| MCV | 92.0 | fl | |
| MCH | 29.6 | pg | |
| MCHC | 322 | g/l | |
| CHCM | 322 | g/l | |
| RDW | 14.9 | % | Anisocytosis + |
| HDW | 2.29 | g/dl | |
| HYPO | 3.0 | % | |
| HYPER | 0.2 | % | |
| MICRO | 0.6 | % | |
| MACRO | 2.0 | % | |
| RBC fragments | 0.03 | ×10$^{12}$/l | |
| RBC ghosts | 0.01 | ×10$^{12}$/l | |
| RET | 1.26 | % | |
| RET | 56.3 | ×10$^9$/l | |
| CHr | 30.2 | pg | |
| IRF (M + H) | 28.2 | % | |
| PLT | 503 | ×10$^9$/l | |
| MPV | 8.8 | fl | |
| PDW | 49.6 | % | |
| Large platelets | 5 | ×10$^9$/l | |
| MPC | 22.6 | g/dl | |

| Parameter | ×10$^9$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 15.07 | | |
| WBC Perox | 14.60 | | |
| NEUT | 7.16 | | |
| LYMPH | 3.60 | | |
| MONO | 0.85 | | |
| EOS | 3.14 | | |
| BASO | 0.09 | | |
| LUC | 0.24 | | |
| NRBC | 0.00 | | |
| LI | 1.93 | | |
| MPXI | 5.6 | | |
| MN | | 30.9 | |
| PMN | | 68.4 | |
| BLASTS | | 0.3 | |
| Perox HPX | | 1.9 | |
| Perox SAT | | 0.3 | |

Full blood count done on a patient with cough, bronchospasm and diarrhoea. All the haematological parameters are normal with the exception of a moderate leucocytosis and thrombocytosis, the latter attributable mainly to a marked increase in eosinophils, both in terms of percentage and absolute count. Clinical and laboratory assessment had not identified any possible cause of secondary eosinophilia. In the Perox cytogram, the eosinophils form a spherical group in the area where these cells are usually located, because of their intense peroxidase activity that leads to absorption of a proportion of the light so that they appear smaller than neutrophils. In the Baso cytogram, the nuclei of the eosinophils appear in the PMN tail, where, if they are numerous as in this case, they form a dense cloud extending downwards from the central portion of the PMN profile.

**Morphology.** The differential white cell count on the blood film gives very similar values to the instrument counts. The eosinophils are cytologically normal and there are no immature forms present. The platelets are normal and well formed. The diagnosis of idiopathic hypereosinophilic syndrome must be considered provisional, particularly because of the moderate thrombocytosis which indicates the need in this patient for further haematological investigations directed at excluding a myeloproliferative neoplasm.

# References

1. Chusid MJ, Dale DC, West BC, Wolff SM. The hypereosinophilic syndrome. Analysis of fourteen cases with review of the literature. Medicine 1975, 54:1–27.
2. Bain BJ. Chronic eosinophilic leukemia, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. Lyon: IARC, 2008:51–53.
3. Bain BJ. Hypereosinophilia. Curr Opin Hematol 2000, 7:21–25.
4. Bain BJ. Eosinophilic leukemia and idiopathic hypereosinophilic syndrome are mutually exclusive diagnoses. Blood 2004, 104:3826.
5. Brito-Babapulle F. Clonal eosinophilic disorders and the hypereosinophilic syndrome. Blood Rev 1997, 11:129–145.
6. Tefferi A, Patnaik MM, Pardanani A. Eosinophilia: secondary, clonal and idiopathic. Br J Haematol 2006, 133:468–492.
7. Fletcher S, Bain B. Diagnosis and treatment of hypereosinophilic syndromes. Curr Opin Hematol 2007, 14:37–42.
8. Valent P, Gleich GJ, Reiter A, et al. Pathogenesis and classification of eosinophil disorders: a review of recent developments in the field. Expert Rev Hematol 2012, 5:157–176.
9. Valent P, Horny HP, Bochner BS, Haferlach T, Reiter A. Controversies and open questions in the definitions and classification of the hypereosinophilic syndromes and eosinophilic leukemias. Semin Hematol 2012, 49:171–181.
10. Valent P, Klion AD, Horny HP, et al. Contemporary consensus proposal on criteria and classification of eosinophilic disorders and related syndromes. J Allergy Clin Immunol 2012, 130:607–612.
11. Roufosse F, Garaud S, de Leval L. Lymphoproliferative disorders associated with hypereosinophilia. Semin Hematol 49:138–148.
12. Simon HU, Plötz SG, Dummer R, Blaser K. Abnormal clones of T cells producing interleukin-5 in idiopathic eosinophilia. N Engl J Med 1999, 341:1112–1120.
13. Noel P. Eosinophilic myeloid disorders. Semin Hematol 2012, 49:120–127.
14. Brito-Babapulle F. The eosinophilias, including the idiopathic hypereosinophilic syndrome. Br J Haematol 2003, 121:203–223.
15. Mejia R, Nutman TB. Evaluation and differential diagnosis of marked, persistent eosinophilia. Semin Hematol 2012, 49:149–159.
16. Crane MM, Chang CM, Kobayashi MG, Weller PF. Incidence of myeloproliferative hypereosinophilic syndrome in the United States and an estimate of all hypereosinophilic syndrome incidence. J Allergy Clin Immunol 2010, 126:179–181.
17. Bain BJ. Eosinophilic leukaemias and the idiopathic hypereosinophilic syndrome. Br J Haematol 1996, 95:2–9.
18. Akuthota P, Weller PF. Eosinophils and disease pathogenesis. Semin Hematol 2012, 49:113–119.
19. Weller PF, Bubley GJ. The idiopathic hypereosinophilic syndrome. Blood 1994, 83:2759–2779.
20. Luppi M, Marasca R, Morselli M, Barozzi P, Torelli G. Clonal nature of hypereosinophilic syndrome. Blood 1994, 84:349–350.
21. Bain BJ. The significance of ring eosinophils in humans. Br J Haematol 1989, 73:580–581.
22. Pantanowitz L, Balogh K. Charcot-Leyden crystals: pathology and diagnostic utility. Ear Nose Throat J 2004, 83:489–490.
23. Grigoriadis G, Anderson MA, Whitehead S. Charcot-Leyden crystals. Intern Med J 2010, 40:792.
24. Gotlib J. Eosinophilic myeloid disorders: new classification and novel therapeutic strategies. Curr Opin Hematol 2010, 17:117–124.
25. Bain BJ. Relationship between idiopathic hypereosinophilic leukemia, and systemic mastocytosis. Am J Hematol 2004, 76:82–85.
26. Gotlib J, Akin J. Mast cells and eosinophils in mastocytosis, chronic eosinophilic leukemia, and non-clonal disorders. Semin Hematol 2012, 49:128–137.
27. Bain BJ. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGRFA, PDGRFB or FGFR1. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. Lyon: IARC, 2008:68–73.
28. Metzgeroth G, Walz C, Score J, et al. Recurrent finding of the FIP1L1-PDGFRA fusion gene in eosinophilia-associated acute myeloid leukemia and lymphoblastic T-cell lymphoma. Leukemia 2007, 21:1183–1188.
29. Cross NC, Reiter A. Fibroblast growth factor receptor and platelet-derived growth factor receptor abnormalities in eosinophilic myeloproliferative disorders. Acta Haematol 2008, 119:199–206.
30. Bain BJ. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGFR1. Haematologica 2010, 95:696–698.
31. Pardanani A, Brockman SR, Paternoster SF, et al. FIP1L1-PDGFRA fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. Blood 2004, 104:3038–3045.
32. Lyall H, O'Connor S, Clark D. Charcot-Leyden crystals in the trephine biopsy of a patient with a FIP1-L1-PDGFRA-positive myeloproliferative disorder. Br J Haematol 2007, 138:405.
33. Steer EJ, Cross NC. Myeloproliferative disorders with translocations of chromosome 5q31–35: role of the platelet-derived growth factor receptor Beta. Acta Haematol 2002, 107:113–122.