

The contribution of fish models in understanding of blood disorders

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Abstract Human and fish peripheral blood contains erythrocytes or red blood cells, leukocytes or white blood cells and platelets or thrombocytes. Although the fish present the same types of blood cells as humans, studies have highlighted some morphological, ultrastructural and cytochemical differences in blood cells of the two groups of vertebrates. In both human and fish hematological tests are indispensable tools which provide a considerable amount of useful information for evaluation of the general state of health. Although there are limited amount of studies on blood cells in fish, over the years especially *Danio rerio*, has become an powerful model organism for studying human hematological diseases, as anemia, leukemia, myeloproliferative diseases and others human hematological diseases. Thereby, fish are ideal model organisms for studying hematological diseases due to external fertilization, brief development time, large number of embryos and the results can be extrapolated to mammals, including human. Furthermore, *ikaros*, *rag-1*, *rag-2* and *lck* genes that are found in human were observed in *Danio rerio* revealing the spatio-temporal proximity between these groups of vertebrates. Therefore, studies realized on fish can offer essential information about molecular mechanism which causes the appearance of numerous human hematological diseases and discovery of possible therapeutic methods. This review provides a comparative overview of blood cells in fish and humans and describes zebrafish mutants as models of human hematologic disorders.

Keywords: fish, human, blood cells, hematological diseases

Introduction

Peripheral blood of fish and humans contains erythrocytes or red blood cells, leukocytes or white blood cells and platelets or thrombocytes (Dagur and McCoy, 2015; Grant, 2015). Fishes are the most numerous and diverse of all vertebrate groups and due to such a diversity of species, the studies published so far on fish peripheral blood revealed considerable variations in structure, as well as in the function of blood cells of different fish species (Grant, 2015; Fazio, 2019). For both humans and fish, the hematological indices are one of the most important and essential parameters for health assessments. In fish, the changes in these parameters depend on the species, age, as well as the environmental conditions (Vázquez and Guerrero, 2007; Tang et al., 2015). For hematological evaluations of all vertebrate groups are required various techniques, such as the determination of red blood cell count (RBC), white blood cell count (WBC), hematocrit or packed cell volume test (PVC), hemoglobin concentration (Hb), erythrocyte indices, white blood cell differential count and the evaluation of stained peripheral blood smears (Vázquez and Guerrero, 2007).

Moreover, leukocytes and platelets are an essential indicator for establishing health status and in many cases can be useful in assessing the immune system. Also, morphological, cytochemical and ultrastructural studies are important for highlighting the possible immune functions of leukocytes (Taveres-Dias and de Moraes, 2007). Although the fish presents the same types of blood cells which have been found in the peripheral blood of humans, studies have highlighted some differences in blood cells of the two types of vertebrates. The differences can be morphological, ultrastructural or cytochemical.

Although in literature is a limited amount of information related to hematological tests of fish (Grant, 2015), these aquatic vertebrates, especially *Danio rerio*, have become an invaluable vertebrate model for studying and mimicking different human disorders in almost all systems, from hematological, hepatic and brain diseases, to autoimmune and psychiatric disorders. Also, hematopoiesis is highly conserved between human and *Danio rerio*, making this species an attractive model for studying hematopoietic development and blood diseases (Avagyan and Zon, 2016).

There are several characteristics that have made *Danio rerio* a versatile and reliable model of human disease, and particularly blood diseases. The advantages for using this species are small size, rapid sexual maturity, high fecundity, external fertilization and transparency of embryos that allow for direct microscopic observation, cell transplantation and lineage tracing (Shafizadeh and Paw, 2004).

Comparative overview of blood cells in fish and humans

1. Erythrocytes

Erythrocytes or red blood cells are the most abundant cell type in blood of both fish and mammals. Fish erythrocytes have an elliptical or oval shape, slightly eosinophilic cytoplasm and centrally located basophilic nucleus, while erythrocytes found in humans are biconcave discoidal cells and have no nucleus (Fig. 1A-B). Mammals, including human, are the only vertebrates which present anucleated erythrocytes at maturity, in peripheral blood. Most fish species have nucleated erythrocytes, with certain exceptions, including *Mauroliscus muelleri* and representatives from Gonosomatidae family, which have anucleated erythrocytes, this aspect being associated with the reduced size of blood vessels in these species (Grant, 2015). Unlike mammals, the size and morphology of erythrocytes varies between fish species (Kumar, 2016). Erythrocytes found in the peripheral blood of most teleosts species have a diameter between 8 - 15 μm , while erythrocytes of abyssal species can measure up to 15 μm . Moreover, in the peripheral blood of dipnoans there are very large erythrocytes, with a diameter of up to 40 μm (Caloianu and Zarnescu, 1999). In contrast, human erythrocytes are much smaller, measuring between 7.5 - 8.7 μm in diameter (Diez-Silva et al., 2010).

Fish erythrocytes have similar functions of oxygen transport as in mammals, including human. Also, defects in enzymatic, metabolic and cytoskeletal proteins often result in erythrocyte abnormalities that can reproduce human erythrocyte diseases despite the lack of nuclei (Carradice and Lieschke, 2008).

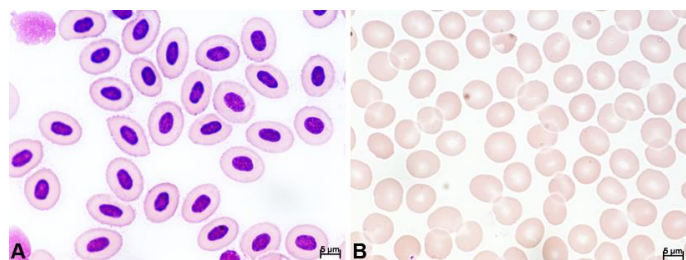


Fig. 1. Fish (*Cyprinus carpio*) (A) and human (B) erythrocytes in Giemsa-stained smears.

2. Leukocytes

Fish leukocytes were classified by same criteria as human leukocytes (Zinkl et al., 1991). Some studies suggest that many of the fish leukocytes have both similar morphology and functions to mammalian leukocytes (Palić et al., 2011). Both leukocytes found in humans and fish are classified into granulocytes and agranulocytes (Zarnescu, 2012; Grant, 2015).

Granular leukocytes found in the peripheral blood of humans and fish include neutrophils, eosinophils and basophils (Grant, 2015; Jablonska and Granot, 2017; Leiding, 2017; Rosales, 2018). Table 1 show granular leukocytes described in fish species.

Table 1 Granulocyte types described in fish species

| FISH SPECIES | AUTHORS |
|----------------------------------|---|
| NEUTROPHILS | |
| <i>Acipenser transmontanus</i> | da Silva et al., 2011 |
| <i>Alburnus alburnus</i> | Ellis, 1977 |
| <i>Anabas testudineus</i> | Acharya and Ackerman, 2014 |
| <i>Aristichthys nobilis</i> | Tavares-Dias, 2006A |
| <i>Astronotus ocellatus</i> | Tavares-Dias, 2006A, B |
| <i>Callorhynchus milii</i> | Hine and Wain, 1988 |
| <i>Carassius auratus</i> | Ellis, 1977; da Silva et al., 2011 |
| <i>Carassius carassius</i> | Zhang et al., 2019 |
| <i>Catostomus commersonii</i> | Barber and Westermann, 1975 |
| <i>Centropomus parallelus</i> | da Silva et al., 2011 |
| <i>Chimaera phantasma</i> | Hine and Wain, 1988 |
| <i>Clarias batrachus</i> | Acharya and Ackerman, 2014 |
| <i>Ctenopharyngodon idella</i> | Zhang et al., 2019; Chen et al., 2019 |
| <i>Cyclopterus lumpus</i> | Rønneseth et al., 2015 |
| <i>Cyprinus carpio</i> | Ellis, 1977; da Silva et al., 2011; Pijanowski et al., 2013 |
| <i>Danio rerio</i> | Grzelak et al., 2017; Torraca and Mostowy, 2017 |
| <i>Dicentrarchus labrax</i> | do Vale et al., 2002 |
| <i>Fundulus heteroclitus</i> | Ellis, 1977 |
| <i>Gadopsis marmoratus</i> | |
| <i>Gadus morhua</i> | Rønneseth et al., 2007 |
| <i>Gymnocypris eckloni</i> | Zheng et al., 2017 |
| <i>Harriotta raleighana</i> | Hine and Wain, 1988 |
| <i>Hoplias malabaricus</i> | Tavares-Dias, 2006A, B |
| <i>Horabagrus brachysoma</i> | Prasad and Charles, 2010 |
| <i>Hydrolagus novaezelandiae</i> | Hine and Wain, 1988 |
| <i>Ictalurus punctatus</i> | Garavini et al., 1981; Ainsworth et al., 1990; Tavares-Dias and de Moraes, 2007 |
| <i>Lates japonicas</i> | Nakada et al., 2014 |
| <i>Lepidosiren paradoxa</i> | Bielek and Strauss, 1993 |
| <i>Megalobrama amblycephala</i> | Chen et al., 2019 |
| <i>Morone saxatilis</i> | da Silva et al., 2011 |

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|--|--|
| <i>Oncorhynchus mykiss</i> | Ellis, 1977; Holland and Rowley, 1998 |
| <i>Pelteobagrus fulvidraco</i> | Chen et al., 2019 |
| <i>Piaractus mesopotamicus</i> | Sado et al., 2014 |
| <i>Pimephales promelas</i> | Palić et al., 2005 |
| <i>Plecoglossus altivelis altivelis</i> | Nakada et al., 2014 |
| <i>Pleuronectes platessa</i> | |
| <i>Rhincodon typus</i> | Dove et al., 2010 |
| <i>Rhinochimaera pacifica</i> | Hine and Wain, 1988 |
| <i>Salmo salar</i> | Ellis, 1977; Haugland et al., 2010 |
| <i>Salmo trutta</i> | Ellis, 1977 |
| <i>Schizothorax prenanti</i> | Fang et al., 2014 |
| <i>Scomber japonicus</i> | Ellis, 1977 |
| <i>Scophthalmus maximus</i> | Chi et al., 2017 |
| <i>Scyliorhinus canicula</i> | Morrow and Pulsford, 1980 |
| <i>Sparus aurata</i> | Meseguer et al., 1994 |
| <i>Tandanus tropicanus</i> | Kelly and Gibson-Kueh, 2015 |
| <i>Tinca tinca</i> | Ellis, 1977 |
| EOSINOPHILS | |
| <i>Acipenser transmontanus</i> | Bianchi et al., 2014 |
| <i>Anabas testudineus</i> | Acharya and Ackerman, 2014 |
| <i>Aristichthys nobilis</i> <i>Astronotus ocellatus</i> | Tavares-Dias, 2006A |
| <i>Callorhynchus milii</i> | Hine and Wain, 1988 |
| <i>Carassius auratus</i> | Ellis, 1977 |
| <i>Catostomus commersonii</i> | Barber and Westermann, 1975 |
| <i>Centropomus parallelus</i> | da Silva et al., 2011 |
| <i>Chimaera phantasma</i> | Hine and Wain, 1988 |
| <i>Clarias batrachus</i> | Acharya and Ackerman, 2014 |
| <i>Ctenopharyngodon idella</i> | Zhang et al., 2019 |
| <i>Cyprinodon variegatus</i> | Ellis, 1977 |
| <i>Cyprinus carpio</i> | Tripathi et al., 2004 |
| <i>Danio rerio</i> | Jagadeeswaran et al., 1999; Grzelak et al., 2017 |
| <i>Dicentrarchus labrax</i> | do Vale et al., 2002 |
| <i>Fundulus heteroclitus</i> | Ellis, 1977 |
| <i>Fundulus majalis</i> | |
| <i>Gymnocypris eckloni</i> | Zheng et al., 2017 |
| <i>Harriotta raleighana</i> | Hine and Wain, 1988 |
| <i>Horabagrus brachysoma</i> | Prasad and Charles, 2010 |
| <i>Hydrolagus novaezelandiae</i> | Hine and Wain, 1988 |
| <i>Ictalurus punctatus</i> | Bianchi et al., 2014 |
| <i>Labrax maculatus</i> | Drury, 1915; Ellis, 1977 |
| <i>Lates japonicas</i> | Nakada et al., 2014 |
| <i>Lepidosiren paradoxa</i> | Bielek and Strauss, 1993 |
| <i>Neoceratodus forsteri</i> | Ellis, 1977 |
| <i>Oreochromis niloticus</i> | Tavares-Dias, 2006A, B |
| <i>Oncorhynchus mykiss</i> | Ellis, 1977; Holland and Rowley, 1998 |
| <i>Oncorhynchus tshawytscha</i> | Ellis, 1977 |
| <i>Piaractus mesopotamicus</i> | Sado et al., 2014 |
| <i>Pimephales promelas</i> | Palić et al., 2005 |
| <i>Rhincodon typus</i> | Dove et al., 2010 |
| <i>Rhinochimaera pacifica</i> | Hine and Wain, 1988 |

| | |
|--|------------------------------------|
| <i>Salmo salar</i> | Ellis, 1977 |
| <i>Scyliorhinus canicula</i> | Morrow and Pulsford, 1980 |
| <i>Sorubim lima</i> | Bianchi et al., 2014 |
| <i>Sparus aurata</i> | Meseguer et al., 1994 |
| <i>Takifugu rubripes</i> | Nakada et al., 2014 |
| BASOPHILS | |
| <i>Acipenser transmontanus</i> | Bianchi et al., 2014 |
| <i>Carassius auratus</i> | Ellis, 1977; da Silva et al., 2011 |
| <i>Carassius carassius</i> | Nakada et al., 2014 |
| <i>Carassius vulgaris</i> | Ellis, 1977 |
| <i>Ctenopharyngodon idella</i> | Zhang et al., 2019 |
| <i>Cyprinus carpio</i> | Ellis, 1977; Tripathi et al., 2004 |
| <i>Cyclopterus lumpus</i> | Rønneseth et al., 2015 |
| <i>Ictalurus punctatus</i> | Bianchi et al., 2014 |
| <i>Lates japonicas</i> | Nakada et al., 2014 |
| <i>Lepidosiren paradoxa</i> | Bielek and Strauss, 1993 |
| <i>Leuciscus leuciscus</i> | Ellis, 1977 |
| <i>Horabagrus brachysoma</i> | Prasad and Charles, 2010 |
| <i>Morone saxatilis</i> | Bianchi et al., 2014 |
| <i>Neoceratodus forsteri</i> <i>Oncorhynchus gorboscha</i> <i>Oncorhynchus keta</i> <i>Oncorhynchus nerka</i> | Ellis, 1977 |
| <i>Rhincodon typus</i> | |
| <i>Salmo irideus</i> | |
| <i>Scyliorhinus canicula</i> | |
| <i>Sorubim lima</i> | Bianchi et al., 2014 |
| <i>Takifugu rubripes</i> <i>Takifugu vermicularis</i> | Nakada et al., 2014 |

Neutrophils represent the largest fraction among granular leukocytes found in peripheral blood of humans and fish. In human the percentage of neutrophils is about 65% of blood leukocytes, while the numbers of circulating neutrophils reported in fish vary. For instance, neutrophils proportion in *Salmo trutta* vary between 0-25% and in *Carassius auratus* the percentage of neutrophils is about 1-12% of total leukocytes (Ellis, 1977). In fish, this type of granulocyte has been described as heterophil and neutrophil. Neutrophil in fish has a similar appearance to the Romanowsky-stained reptile or avian heterophil and may have the cytochemical properties of a mammalian neutrophil (Ueda et al., 2001; Carradice and Lieschke, 2008; Grant, 2015). Morrow and Pulsford (1980) described four granulocyte types (I to IV or G₁, G₂, G₃ and G₄) in *Scyliorhinus canicula* according to the morphology of the cytoplasmic granules. Recently, elasmobranch granulocytes were classified using avian nomenclature, such as heterophils and eosinophils, but this nomenclature works in species that have only these two types of granulocytes (Carnezim and Marcos, 2020), while for teleost the suggested term for this type of granulocyte is neutrophil (Ueda et al., 2001; Grant, 2015; Kumar, 2016).

In fish, neutrophils have very different sizes, measuring on average 10-12 µm in diameter (Tripathi et al., 2004). Instead, neutrophils present in the circulating blood of

humans are larger, measuring between 12-15 μm in diameter (Kolaczowska and Kubes, 2013). Outside the blood, neutrophils were found especially in loose connective tissue in humans and in kidneys and spleen in fish (Palić et al., 2011). Neutrophils number increase, both in fish and humans, in inflammatory lesions highlighting their phagocytic, bactericidal activity and chemotactic functions (Styrt, 1989; Palić et al., 2011; Kolaczowska and Kubes, 2013).

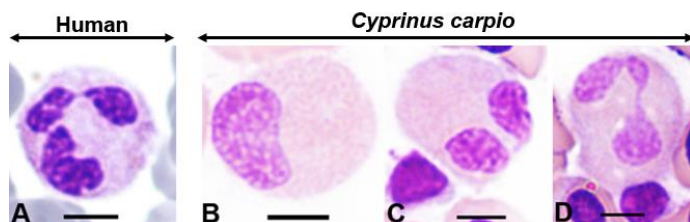


Fig. 2. Human (A) and fish (*Cyprinus carpio*) (B-D) neutrophils in Giemsa-stained smears. Bar 5 μm .

Structurally, in both fish and humans neutrophils are spherical, nucleated cells (Fig. 2A-D). Fish neutrophils have an eccentric nucleus which varied in shape from round, oval, kidney-shaped to lobed (usually with two to three lobes) (Fig. 2B-D). In humans, nucleus is segmented, consisting of two-five nuclear lobes (Fig. 2A). In contrast to human where it is also used the term polymorphonuclear leukocytes (PMN) for neutrophils, in fish because in certain species the eccentric nucleus is oval or round this term would be inappropriate, though in some species (Salmonidae) the neutrophils also possess lobed nuclei (Ellis, 1977).

In fish neutrophils cytoplasm is abundant and has numerous fine azurophilic granules which are stained in light red or violet on blood smears (Ueda et al., 2001). The cytoplasm of human granulocytes is rich in granules and secretory vesicles (Kolaczowska and Kubes, 2013; Leiding, 2017). In both human and fish, the cytoplasm of neutrophils contains different types of granules with many antimicrobial substances, as lysozyme, which are released into phagosomes or outside the cell during degranulation (Palić et al., 2011). Unlike human neutrophils, chemotaxis has not been demonstrated convincingly for fish neutrophils (Ellis, 1977).

Ultrastructurally, the cytoplasm of fish and humans neutrophils contains Golgi apparatus, mitochondria, endoplasmic reticulum, glycogen granules as well as specific granules (Fänge, 1992; Kolaczowska and Kubes, 2013; Leiding, 2017). The cytoplasm of fish neutrophils has a large number of small (0.2 and 0.5 μm), round or elongated electron-dense granules (da Silva et al., 2011) that can be homogeneous, or contain fibrillary inclusions at *Pleuronectes platessa* (Ferguson, 1976) or aciform crystalloids at *Cyprinus carpio* (Tripathi et al., 2004).

In the cytoplasm of human neutrophils actin microfilaments and microtubules has been described. Also, human neutrophils have three types of granules formed sequentially during their maturation. Azurophilic

or primary granules with myeloperoxidase, specific or secondary granules with lactoferrin and tertiary granules that store matrix metalloproteinase 9 (Kolaczowska and Kubes, 2013; Leiding, 2017). Small and large granules in neutrophils of *Esox lucius* structurally resemble primary and secondary granules of mammalian neutrophils, including human (Savage, 1983).

Histochemically, human (Table 2) and fish neutrophils (Table 3) have different staining properties. Moreover, fish neutrophils have different staining properties from one species to another (Palić et al., 2011). However, fish neutrophils studied over time are positive for many staining techniques, as PAS (Periodic Acid Schiff) (Fig. 3A), Sudan Black (Fig. 3B), peroxidase (Fig. 3C), bromophenol blue and naphthol AS-D chloroacetate esterase.

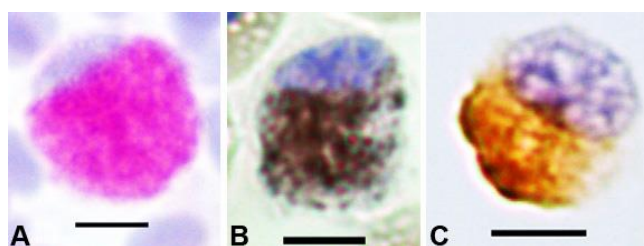


Fig. 3. Fish (*Cyprinus carpio*) neutrophils stained with PAS (A), Sudan Black (B) and peroxidase (C). Bar 5 μm .

Table 2 Cytochemical characteristic of human granulocytes

| Cytochemical reaction | Granulocyte types | Reaction +/- | Authors |
|-----------------------|-------------------|--------------|---------------------------------------|
| Toluidine blue | Neutrophils | - | Bogder and Newton, 1987 |
| | Eosinophils | - | Shoham et al, 1974; Liso et al., 1977 |
| | Basophils | + | Parwaresch, 1976 |
| AS-D | Neutrophils | + | Bogder and Newton, 1987 |
| | Eosinophils | + | Liso et al., 1977 |
| | Basophils | - | Parwaresch, 1976 |
| Nonspecific esterase | Neutrophils | - | Bogder and Newton, 1987 |
| | Basophils | + | Parwaresch, 1976 |
| Acid phosphatase | Neutrophils | - | Bogder and Newton, 1987 |
| | Eosinophils | + | Shoham et al, 1974; Liso et al., 1977 |
| | Basophils | + | Parwaresch, 1976 |
| Alkaline phosphatase | Neutrophils | - | Bogder and Newton, |

| | | | |
|-------------|-------------|-----|---|
| | | | 1987 |
| | Eosinophils | - | Liso et al., 1977 |
| | Basophils | - | Parwaresch, 1976 |
| MPO | Neutrophils | - | Bogder and Newton, 1987 |
| | | + | Parwaresch, 1976; Parmley et al., 1987 |
| | Eosinophils | + | Parwaresch, 1976 |
| | Basophils | + | Parwaresch, 1976 |
| PAS | Neutrophils | + | Parwaresch, 1976; Bogder and Newton, 1987 |
| | Eosinophils | + | Shoham et al., 1974; Liso et al., 1977 |
| | Basophils | - | Parwaresch, 1976 |
| Peroxidase | Neutrophils | + | Bogder and Newton, 1987; Parmley et al., 1987; Saito et al., 1998 |
| | Eosinophils | + | Shoham et al., 1974; Liso et al., 1977 |
| | Basophils | -/+ | Parwaresch, 1976 |
| Sudan Black | Neutrophils | - | Bogder and Newton, 1987 |
| | Eosinophils | + | Parwaresch, 1976; Liso et al., 1977 |
| | Basophils | + | Parwaresch, 1976 |

AS-D - naphthol AS-D chloroacetate esterase; MPO - Myeloperoxidase; PAS - Periodic Acid Schiff; + positive reaction; - negative reaction.

Eosinophils compared to neutrophils are less common in both fish and human (Wen and Rothenberg, 2016). In human, eosinophils form 1-3% of blood leukocytes, while in fish they represent between 2 - 8% of the total leukocytes population (Ellis, 1977). In some fish species this type of granulocyte has not been reported. In both human and fish, eosinophils may be involved in defense against inflammation and parasitic infections through phagocytosis (Grant, 2015).

In humans, the number of eosinophils increases under pathological conditions such as allergic reactions and parasitic worm infections (Hogan et al., 2008). In

Labridae species starvation caused disappearance of eosinophils from peripheral blood and these observations might resemble the disappearance of eosinophils in mammals caused by hydrocortisone, while in *Mustelus* spp. was reported the opposite. However, it was reported that stressful experience as capture, crowding and starvation resulted in an increase in blood eosinophils (Ellis, 1977).

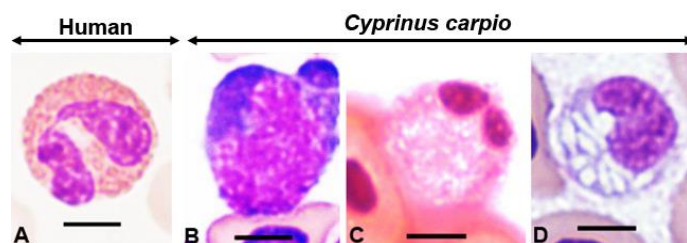


Fig. 4. Human (A) and fish (*Cyprinus carpio*) (B-D) eosinophils in Giemsa-stained smears. Bar 5 µm.

Structurally, human (Fig. 4A) and fish eosinophils (Fig. 4B-D) are round cells that contain cytoplasmic granules with affinity for acid dyes (Bianchi et al., 2014; Ravin and Loy, 2016; Kumar, 2016). While human eosinophils have a bilobed nucleus (Fig. 4A), fish eosinophils may have an eccentric, unsegmented nucleus or a bilobate nucleus (Fig. 4B-C).

Ultrastructurally, a very large diversity of eosinophils has been reported in fish. Both human and fish eosinophils nucleus has agglomerations of heterochromatin. In cytoplasm are Golgi apparatus, vesicles, free ribosomes, mitochondria and rough endoplasmic reticulum (Vázquez and Guerrero, 2007; Zheng et al., 2017; Melo and Weller, 2018). Generally, fish eosinophils have relatively large granules with a diameter about 0.8 µm (Fänge, 1992) while human eosinophils have azurophilic and specific granules that measure up to 0.5 µm (Melo and Weller, 2018). Azurophilic granules or lysosomes contained acid hydrolases, as well as hydrolytic enzymes with role in destroying parasites, while specific granules contain many types of proteins, as major basic protein, cationic protein, peroxidase, eosinophil-derived neurotoxin, arylsulfatase, collagenase and cathepsins, all having a role in defending the body against infections (Denburg, 1998). In bony fish it was discovered that eosinophils granules compared to those of mammals, including human, do not possess crystalline inclusions (Fänge, 1992). Sometimes, at some fish species like *Cyprinus carpio*, eosinophils with large granules (Fig. 4D) were identified (our unpublished data). These large granules form as a result of the granules “melting” and running together like in *Labrax maculatus* (Drury, 1915).

From a cytochemical point of view, eosinophils show similarities between humans (Table 2) and some fish species (Table 3). Thus, both human and fish eosinophils are positive for naphthol AS-D chloroacetate esterase, acid phosphatase and PAS. Unlike human eosinophils, those of some fish species are positive for α -naphthyl butyrate esterase and alkaline phosphatase.

Basophils are the third type of granulocyte found in peripheral blood of fish and humans, but in fish the presence of basophils is very controversial (Ueda et al., 2001). It has been reported that these granulocytes are very rare or even absent in peripheral blood of some fish species (Table 1). In human, basophils constitute about 0.5 – 1% of circulating leukocytes (Ishikawa et al., 2009), while in fish their percentage may be different depending on the species, for instance in *Neoceratodus forsteri* the proportion of basophils is 1%, in *Carassius vulgaris* 2% and in *Cyprinus carpio* 9% of total leukocytes (Ellis, 1977). Basophils found in peripheral blood of fish are the smallest granular leukocytes, having a diameter about 8-10 μm , while human basophils measure between 12-15 μm in diameter (Tripathi et al., 2004).

Both, human (Fig. 5A) and fish (Fig. 5B) basophils are spherical cells with a cytoplasm rich in basophilic granules of different sizes, which often masked the nucleus (Fig. 5A-B). Fish basophils have an spherical nucleus while human basophils have a polymorphous nucleus, often S shaped.

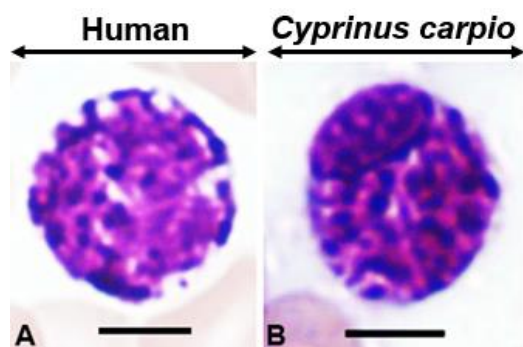


Fig. 5. Human (A) and fish (*Cyprinus carpio*) (B) basophils in Giemsa-stained smears. Bar 5 μm .

Human and fish basophils have mitochondria and ribosomes (Tripathi et al., 2004; Dvorak, 2005). Also, in human basophils it has been identified endoplasmic reticulum and glycogen granules scattered throughout the cytoplasm (Tripathi et al., 2004). Moreover, it was highlighted that these granulocytes have fine granules and occasional can be discovered small cytoplasmic vacuoles (Bianchi et al., 2014).

Human basophils have cytoplasmic vesicles and filaments and Golgi apparatus is difficult to be observed. Also, both fish and human basophils release inflammatory mediators, such as histamine through degranulation. Histamine was initially thought to be absent in fish basophils, but Odaka et al. (2018) demonstrated that teleost basophils store histamine in their granules.

From a cytochemical point of view, basophils show similarities between humans (Table 2) and some fish species (Table 3). Thus, both human and fish basophils are positive for acid phosphatase, peroxidase and Sudan Black. Unlike human basophils, those of some fish species are positive for naphthol AS-D chloroacetate

esterase, alkaline phosphatase, PAS and bromophenol blue.

Agranular leukocytes or agranulocytes have been identified in both human and fish and include lymphocytes and monocytes.

Human and fish **lymphocytes** have the highest percentage of total leukocytes. In fish the percentage of lymphocytes is about 85% of total leukocyte population (Vázquez and Guerrero, 2007), while in human the percentage of lymphocytes is about 42% of total leukocytes (Hulstaert et al., 1994). Counts of lymphocyte and lymphocyte subset are a great value to ensure the functionality of the immune system and to detect immunodeficiency, viral infections and other infectious diseases that can lead to abnormal levels of lymphocyte (Liu et al., 2020). Both human and fish lymphocytes are spherical cells with oval or round nucleus (Fig. 6). Human lymphocytes are classified into T cells, matured in the thymus, that are responsible for cell mediated immunity, B cells responsible for antibody production (Ellis, 1977; Larosa and Orange, 2008) and natural killer cells (Larosa and Orange, 2008). Fish possess lymphocyte populations that are analogous to T cells, B cells and natural killer cells of human (Uribe et al., 2011). Both human and fish lymphocytes have important functions in host defense against bacteria, microbes and tumors (Larosa and Orange, 2008; Scapigliati et al., 2018). Also, lymphocytes are responsible for adaptive responses, but in literature is reported that subpopulations of mammalian lymphocytes, including human, behave like innate-like cells, engaging non-self rapidly and without antigen presentation (Scapigliati et al., 2018).

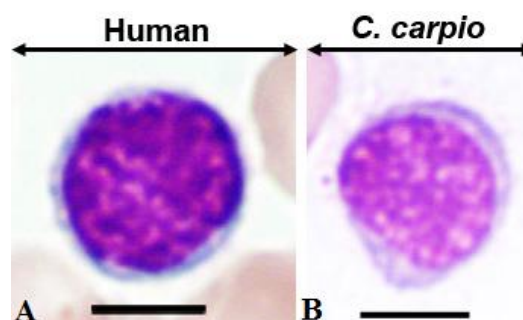


Fig. 6. Human (A) and fish (*Cyprinus carpio*) (B) lymphocyte in Giemsa-stained smears. Bar 5 μm .

Human and fish **monocytes** are the largest types of leukocyte from peripheral blood (Zhou et al., 2012; Grant, 2015). Some fish species have a relatively low percentage of monocytes (Zhou et al., 2012). Morphological, fish and human monocytes are spherical cells and have large, kidney-shaped or horseshoe-shaped nucleus (Fig. 7). In inflammatory conditions human and fish monocytes become macrophages. Macrophage CSF1 (Colony Stimulating Factor 1) have an essential function in macrophage growth and differentiation in both vertebrates. Also, human and fish monocytes are important immune cells and the inflammatory response of macrophages have an essential role against diverse

pathogens. Phagocytosis is the essential role of macrophages and most studies suggest that in fish as found in human, monocytes, macrophages and neutrophils are the main phagocytic cells (Lu and Chen, 2019). Moreover, it was discovered that the monocytes of some fish species resembled human monocytes histochemically and possess a few fine and scattered granules which are positive for PAS and acid phosphatase (Ellis, 1977).

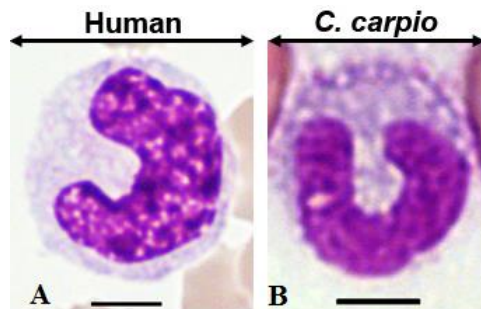


Fig. 7. Human (A) and fish (*Cyprinus carpio*) (B) monocyte in Giemsa-stained smears. Bar 5 µm.

3. Thrombocytes

In literature is reported that thrombocytes in fish, amphibians, reptiles and birds are analogues of platelets in mammals and also human (Stosik et al., 2019). The difference between fish and mammalian thrombocytes, including human is that fish thrombocytes are nucleated cells (Fig. 8B), while human thrombocytes are anucleated fragments of cytoplasm (Fig. 8A). Fish thrombocytes have variable shapes depending on fish species, but in general they are round or elliptical cells with a centrally located dark colored nucleus (Kumar, 2016). Cytoplasm is clear and may have slightly eosinophilic granules. Human thrombocytes are anucleated fragments of cytoplasm which contain on their surface proteins that allow them to join platelets together (Graham, 2002).

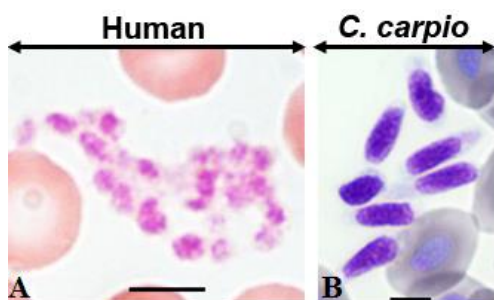


Fig. 8. Human (A) and fish (*Cyprinus carpio*) (B) thrombocytes in Giemsa-stained smears. Bar 5 µm.

In fish, electron microscopy studies reveal a canalicular structure similar to human platelets and pseudopodia-like projections in aggregates (Carradice and Lieschke, 2008). Both human and fish thrombocytes have a major role in maintaining vascular integrity, hemostasis and in

aggregation and release reactions in cases of blood vessel damage and in the immune response development (Thomas and Storey, 2015; Stosik et al., 2019). Moreover, it was discovered that *Danio rerio* thrombocytes function analogously to human platelets in adherence, secretion and aggregation (Carradice and Lieschke, 2008).

Human hematological diseases studied on fish

Hematological tests represents in both human and fish indispensable tools which provide a considerable amount of useful information for evaluation of the general state of health of these two types of vertebrates (Kelly and Gibson-Kueh, 2015). Variation in hematological indices in fish depends of species, age and environmental conditions (Vázquez and Guerrero, 2007; Kelly and Gibson-Kueh, 2015; Tang et al., 2015). Although in literature is a limited amount of information related to hematological tests of fish (Grant, 2015), especially *Danio rerio*, are considered an excellent model for studying and mimicking different human hematological diseases, as leukemia, myeloproliferative diseases and others human hematological diseases (Table 4). Although the origin of blood cells is different in *Danio rerio* and human, hematopoiesis is preserved between the two groups of vertebrates (He et al., 2014; Zizioli et al., 2019). Thereby, *Danio rerio* is an ideal organism for studying hematopoiesis, immune system and hematological diseases and the results can be extrapolated to mammals, including human, due to genetic and molecular similarities (Colucci-Guyon et al., 2011). *Danio rerio* small sizes and high reproductive capacity allowing obtaining a large-scale genetic profile of mutants for every hematological disease (Brownlie et al., 1998). Interest in *Danio rerio* dates to the 1930s and this species have contributed to hematologic research for more than 90 years. The genetic experimental approaches began with the collection of *Danio rerio* mutants that have hematopoietic defects, mostly anemia (Carradice and Lieschke, 2008). The zebrafish genome, first published in 2002, and later modified and expanded in 2013, showed that 70% of human genes have a fish ortholog (Avagyan and Zon, 2016). Also, following comparisons between fish and human hematopoiesis and lymphopoiesis has been proven that genes underlying blood cells development are highly conserved in the evolution. Specific genes, including *ikaros*, *rag-1*, *rag-2* and *lck* which are found in human have been discovered in the zebrafish genome, this fact highlighting spatio-temporal proximity between these groups of vertebrates. Thus, studies on fish can offer essential information about molecular mechanisms which causes human neoplasms (Chen et al., 2007; Gore et al., 2018).

Table 3. Cytochemical characteristics of fish granulocytes

| Cytochemical reaction | Fish species | Granulocyte types | Reaction +/- | Authors |
|----------------------------------|-------------------------------------|-------------------------------------|--------------------------|--------------------------|
| ANAE | <i>Callorhynchus milii</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Carassius auratus</i> | Neutrophils, basophils | - | Zhang et al., 2019 |
| | <i>Chimaera phantasma</i> | Neutrophils, eosinophils | + | Hine and Wain, 1988 |
| | <i>Ctenopharyngodon idella</i> | Neutrophils, basophils | - | Zhang et al., 2019 |
| | | Neutrophils | - | Chen et al., 2019 |
| | <i>Hydrolagus novaezelandiae</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Horabagrus brachysoma</i> | Neutrophils, eosinophils, basophils | - | Prasad and Charles, 2010 |
| | <i>Megalobrama amblycephala</i> | Neutrophils | - | Chen et al., 2019 |
| | <i>Pelteobagrus fulvidraco</i> | Neutrophils | - | |
| | <i>Pimephales promelas</i> | Neutrophils | - | Palić et al., 2005 |
| | <i>Rhinochimaera pacifica</i> | Neutrophils, eosinophils | + | Hine and Wain, 1988 |
| <i>Schizothorax prenanti</i> | Neutrophils | + | Fang et al., 2014 | |
| ANBE | <i>Carassius auratus</i> | Neutrophils, basophils | - | Zhang et al., 2019 |
| | <i>Callorhynchus milii</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Ctenopharyngodon idella</i> | Neutrophils, basophils | - | Zhang et al., 2019 |
| | <i>Chimaera phantasma</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Rhinochimaera pacifica</i> | Neutrophils, eosinophils | + | |
| AS-D | <i>Carassius auratus</i> | Neutrophils, basophils | + | Zhang et al., 2019 |
| | <i>Callorhynchus milii</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Cyprinus carpio</i> | Eosinophils | + | Tripathi et al., 2004 |
| | | Basophils | - | |
| | <i>Chimaera phantasma</i> | Neutrophils | + | Hine and Wain, 1988 |
| | | Eosinophils | - | |
| | <i>Ctenopharyngodon idella</i> | Neutrophils, basophils | + | Zhang et al., 2019 |
| | <i>Dicentrarchus labrax</i> | Neutrophils | - | do Vale et al., 2002 |
| <i>Horabagrus brachysoma</i> | Neutrophils, eosinophils, basophils | - | Prasad and Charles, 2010 | |
| <i>Hydrolagus novaezelandiae</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 | |
| Acid phosphatase | <i>Carassius auratus</i> | Neutrophils, basophils | + | Zhang et al., 2019 |
| | <i>Callorhynchus milii</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Chimaera phantasma</i> | Neutrophils, eosinophils | + | |
| | <i>Ctenopharyngodon idella</i> | Neutrophils, basophils | + | Zhang et al., 2019 |
| | <i>Dicentrarchus labrax</i> | Neutrophils | + | do Vale et al., 2002 |
| | <i>Harriotta raleighana</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Hydrolagus novaezelandiae</i> | Neutrophils, eosinophils | + | |
| | <i>Rhinochimaera pacifica</i> | Neutrophils, eosinophils | - | |
| | <i>Sparus aurata</i> | Neutrophils | - | Meseguer et al., 1994 |
| | | Eosinophils | + | |
| <i>Schizothorax prenanti</i> | Neutrophils | + | Fang et al., 2014 | |
| Alkaline phosphatase | <i>Acipenser transmontanus</i> | Neutrophils | + | da Silva et al., 2011 |
| | | Eosinophils | + | Bianchi et al., 2014 |
| | <i>Aristichthys nobilis</i> | Neutrophils | - | Tavares-Dias, 2006 A |
| | <i>Astronotus ocellatus</i> | Neutrophils, eosinophils | - | |
| | <i>Callorhynchus milii</i> | Neutrophils | + | Hine and Wain, 1988 |
| Eosinophils | | - | | |

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|----------------------------------|---|-------------------------------------|----------------------------------|--------------------------|
| | <i>Carassius auratus</i> | Neutrophils | + | da Silva et al., 2011 |
| | | Neutrophils, basophils | + | Zhang et al., 2019 |
| | <i>Centropomus parallelus</i> | Neutrophils, eosinophils | - | da Silva et al., 2011 |
| | <i>Cyclopterus lumpus</i> | Neutrophils | + | Rønneseth et al., 2015 |
| | <i>Cyprinus carpio</i> | Neutrophils | - | da Silva et al., 2011 |
| | | Basophils | - | Tripathi et al., 2004 |
| | <i>Chimaera phantasma</i> | Neutrophils | + | Hine and Wain, 1988 |
| | | Eosinophils | - | |
| | <i>Ctenopharyngodon idella</i> | Neutrophils, basophils | + | Zhang et al., 2019 |
| | <i>Gymnocypris eckloni</i> | Neutrophils | - | Zheng et al., 2017 |
| | | Eosinophils | - | Tripathi et al., 2004 |
| | <i>Harriotta raleighana</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Hoplias malabaricus</i> | Neutrophils | - | Tavares-Dias, 2006 A |
| | <i>Horabagrus brachysoma</i> | Neutrophils, eosinophils, basophils | - | Prasad and Charles, 2010 |
| | <i>Hydrolagus novaezelandiae</i> | Neutrophils | + | Hine and Wain, 1988 |
| | | Eosinophils | - | |
| | <i>Ictalurus melas</i> | Neutrophils | + | Garavini et al., 1981 |
| | <i>Lates japonicas</i> | Neutrophils, eosinophils | - | da Silva et al., 2011 |
| | <i>Morone saxatilis</i> | Neutrophils | - | |
| | <i>Plecoglossus altivelis altivelis</i> | Neutrophils | + | Nakada et al., 2014 |
| | <i>Pleuronectes platessa</i> | Neutrophils | + | |
| | <i>Rhinochimaera pacifica</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Salmo irideus</i> | Basophils | + | Nakada et al., 2014 |
| <i>Schizothorax prenanti</i> | Neutrophils | - | Fang et al., 2014 | |
| <i>Sparus aurata</i> | Neutrophils | - | Meseguer et al., 1994 | |
| | Eosinophils | + | | |
| <i>Takifugu vermicularis</i> | Basophils | - | Nakada et al., 2014 | |
| Arylsulphatase | <i>Dicentrarchus labrax</i> | Neutrophils | + | do Vale et al., 2002 |
| MPO | <i>Cyclopterus lumpus</i> | Neutrophils | + | Rønneseth et al., 2015 |
| | <i>Gadus morhua</i> | Neutrophils | + | Rønneseth et al., 2007 |
| | <i>Pimephales promelas</i> | Neutrophils | + | Palić et al., 2005 |
| | <i>Scophthalmus maximus</i> | Neutrophils | + | Chi et al., 2017 |
| Oil Red | <i>Schizothorax prenanti</i> | Neutrophils | + | Fang et al., 2014 |
| PAS | <i>Carassius auratus</i> | Neutrophils, basophils | + | Zhang et al., 2019 |
| | <i>Callorhynchus milii</i> | Neutrophils, eosinophils | + | Hine and Wain, 1988 |
| | <i>Chimaera phantasma</i> | Neutrophils, eosinophils | + | |
| | <i>Ctenopharyngodon idella</i> | Neutrophils, basophils | + | Zhang et al., 2019 |
| | | Neutrophils | + | Chen et al., 2019 |
| | <i>Cyprinus carpio</i> | Eosinophils | - | Tripathi et al., 2004 |
| | | Basophils | + | |
| | <i>Gymnocypris eckloni</i> | Neutrophils | + | |
| | <i>Harriotta raleighana</i> | Neutrophils | + | Hine and Wain, 1988 |
| | | Eosinophils | - | |
| <i>Hydrolagus novaezelandiae</i> | Neutrophils, eosinophils | + | | |
| <i>Ictalurus punctatus</i> | Neutrophils | + | Tavares-Dias and de Moraes, 2007 | |

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|------------------------------|---|--------------------------|-----------------------|-----------------------------|
| | <i>Oncorhynchus mykiss</i> | Neutrophils | + | Ellis, 1977 |
| | <i>Pimephales promelas</i> | Neutrophils | - | Palić et al., 2005 |
| | <i>Rhinochimaera pacifica</i> | Neutrophils, eosinophils | + | Hine and Wain, 1988 |
| | <i>Schizothorax prenanti</i> | Neutrophils | + | Fang et al., 2014 |
| | <i>Scomber japonicus</i> | Neutrophils | + | Ellis, 1977 |
| | <i>Tandanus tropicanus</i> | Neutrophils | + | Kelly and Gibson-Kueh, 2015 |
| Peroxidase | <i>Acipenser transmontanus</i> | Eosinophils | - | Bianchi et al., 2014 |
| | <i>Aristichthys nobilis</i> | Neutrophils | - | Tavares-Dias, 2006 A |
| | <i>Astronotus ocellatus</i> | Neutrophils | + | |
| | | Eosinophils | - | |
| | <i>Callorhynchus milii</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Carassius auratus</i> | Neutrophils | - | da Silva et al., 2011 |
| | | Neutrophils, basophils | + | Zhang et al., 2019 |
| | <i>Catostomus commersonii</i> | Neutrophils | - | Barber and Westermann, 1975 |
| | <i>Chimaera phantasma</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Ctenopharyngodon idella</i> | Neutrophils, basophils | + | Zhang et al., 2019 |
| | <i>Cyprinus carpio</i> | Neutrophils | + | da Silva et al., 2011 |
| | | Eosinophils | - | Tripathi et al., 2004 |
| | | Basophils | + | |
| | <i>Dicentrarchus labrax</i> | Neutrophils | + | do Vale et al., 2002 |
| | | Eosinophils | - | |
| | <i>Gymnocypris eckloni</i> | Eosinophils | - | Tripathi et al., 2004 |
| | <i>Harriotta raleighana</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Horabagrus brachysoma</i> | Neutrophils | + | Prasad and Charles, 2010 |
| | | Eosinophils, basophils | - | |
| | <i>Hydrolagus novaezelandiae</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| | <i>Ictalurus melas</i> | Neutrophils | + | Garavini et al., 1981 |
| | <i>Ictalurus punctatus</i> | Eosinophils | - | Bianchi et al., 2014 |
| | <i>Lates japonicas</i> | Eosinophils | - | Nakada et al., 2014 |
| | <i>Megalobrama amblycephala</i> | Neutrophils | + | Chen et al., 2019 |
| | <i>Morone saxatilis</i> | Neutrophils | - | da Silva et al., 2011 |
| | <i>Pelteobagrus fulvidraco</i> | Neutrophils | + | Chen et al., 2019 |
| | <i>Plecoglossus altivelis altivelis</i> | Neutrophils | + | Nakada et al., 2014 |
| | | Neutrophils | + | |
| | <i>Pleuronectes platessa</i> | Neutrophils | + | |
| | <i>Rhinochimaera pacifica</i> | Neutrophils, eosinophils | - | Hine and Wain, 1988 |
| <i>Salmo irideus</i> | Basophils | + | Nakada et al., 2014 | |
| <i>Schizothorax prenanti</i> | Neutrophils | + | Fang et al., 2014 | |
| <i>Sparus aurata</i> | Neutrophils | - | Meseguer et al., 1994 | |
| | Eosinophils | + | | |
| <i>Takifugu rubripes</i> | Eosinophils | - | Nakada et al., 2014 | |
| <i>Takifugu vermicularis</i> | Basophils | - | | |
| Sudan Black | <i>Acipenser transmontanus</i> | Neutrophils | - | da Silva et al., 2011 |

| | | | | |
|--|-------------------------------|------------------------|---|-----------------------|
| | | Eosinophils | - | Bianchi et al., 2014 |
| | <i>Carassius auratus</i> | Neutrophils | + | da Silva et al., 2011 |
| | <i>Centropomus parallelus</i> | Neutrophils | + | |
| | | Eosinophils | - | |
| | <i>Cyprinus carpio</i> | Eosinophils, basophils | - | Tripathi et al., 2004 |
| | <i>Ictalurus punctatus</i> | Eosinophils | - | Bianchi et al., 2014 |
| | <i>Morone saxatilis</i> | Neutrophils | + | da Silva et al., 2011 |
| | <i>Oncorhynchus mykiss</i> | Neutrophils | + | Ellis, 1977 |
| | <i>Oreochromis niloticus</i> | Eosinophils | - | Tavares-Dias, 2006 A |
| | <i>Pimephales promelas</i> | Neutrophils | + | Palić et al., 2005 |
| | <i>Sorubim lima</i> | Eosinophils, basophils | + | Bianchi et al., 2014 |

ANAE = α -naphthyl acetate esterase; ANBE = α -naphthyl butyrate esterase; AS-D = naphthol AS-D chloroacetate esterase; PAS = Periodic Acid Schiff; + = positive reaction; - = negative reaction.

Hematopoietic mutants have diverse hematopoietic defects, being models for hereditary blood diseases and provide tools for drug-target discovery (Carradice and Lieschke, 2008). For instance, *Danio rerio* was used for tumor cells transplantation to allow quantification of leukemic cells and for understanding their molecular mechanism of production, capacity to renew and drug resistance. Thus, human leukemia cell lines can be transplanted in *Danio rerio* embryos for generating *in vivo* xenograft models for experimental leukemia therapy (Zeisig et al., 2012; Harrison et al., 2016). It has been proven that morphology, molecular mechanisms of induction and manifestation of malignant tumors in *Danio rerio* are very similar with those observed in mammals, including human. Most tumors induced in this species reveal the capacity of invasive growth and metastasis of oncogenes (Mizgirev and Revskoy, 2010).

Fish models for erythrocyte diseases

Anemia, defined as a decreased quantity of erythrocytes, is a major source of morbidity and mortality worldwide. There are many forms of anemia, each with its own cause. Through large-scale mutagenesis, several models of human anemias have been established in zebrafish, enabling to better understand the pathogenesis and develop novel treatments for this disease.

Congenital dyserythropoietic anemias are rare hereditary disorders characterized by anemia, inefficient erythropoiesis and abnormal erythroblasts and erythrocytes, being one of the human diseases studied in zebrafish (Moreno-Carralero et al., 2018). *Retsina* mutant (*ret*) used for studying this disease has a specific defect in cell division associated with dyserythropoiesis. Erythroblast of *ret* mutants are binuclear, and exhibit apoptosis, due to segregation errors in mitosis (Paw et al., 2003).

Merlot (*mot*) and *chablis* (*cha*) are mutants used to study **severe hemolytic anemia**, an autoimmune human disease characterized by loss of integrity and deformation of the erythrocyte cell membrane. Protein

4.1 is one of the structural multifunctional proteins from erythrocytes membranes that are indispensable for maintaining the morphology, integrity and mechanical stability and deficiency of this protein determines the appearance of hemolytic anemia (Shafizadeh et al., 2002).

Diamond-Blackfan anemia is a rare genetic blood disorder characterized by a failure of the bone marrow to produce red blood cells. Disease is caused by mutations in ribosomal proteins (RPs) genes, most often in ribosomal protein small subunit 19 (RPS19), and the activation of *p53* is a common response in this deficiency (Vlachos et al., 2012; Danilova et al., 2018). *Rps29* zebrafish mutant, used for studying this disease includes defects in erythrocyte development and an increase of apoptosis in erythrocytes, demonstrating that *p53* is a mediator of the *Rps29* mutant phenotype. *Rps29* is an important protein in biogenesis and processing of ribosomal RNA and the advantage of using zebrafish is the possibility to compare homozygous and heterozygous mutants, but also for the identification of gene expression which correlates with the *rps29* level (Taylor et al., 2012).

Fanconi anemia is a rare autosomal recessive genetic disorder characterized by a defect in DNA repair mechanisms. Patients with Fanconi anemia have congenital malformations, including leukopenia, thrombocytopenia, microphthalmia, microcephaly and dwarfism (Liu et al., 2003). Zebrafish *Rad51* loss-of-function mutants have been used to study this type of disorder because developed key features of Fanconi anemias, including decreased proliferation and increased hematopoietic stem cell apoptosis and cell progenitors (Botthof et al., 2017).

Microcytic anemia characterized through a decrease in the mean erythrocyte volume, caused by iron deficiency and thalassemia, is associated with reduced hemoglobin level due to insufficient availability of heme or globin (Aydogan et al., 2019). In zebrafish *chianti* mutants (*cia*) hypochromia is caused by a mutation in the blood

specific transferrin receptor 1 gene resulting in the inability of blood cells to absorb iron from the plasma (Wingert et al., 2004).

Patients with **sideroblastic anemia** have ring sideroblasts instead of healthy red blood cells in bone marrow. Sideroblasts are abnormal nucleated erythroblasts with iron deposits accumulated in the mitochondria (Harigae, 2017). The zebrafish *sauternes* (*sau*) mutants carry a mutation in the ALAS-2 (5'-aminolevulinate synthase) gene that causes microcytic, hypochromic anemia and defective heme synthesis. During embryogenesis *sau* mutants have delayed erythroid maturation and abnormal globin gene expression (Brownlie et al., 1998).

Erythropoietic protoporphyria is one type of porphyria. It is an inherited disorder caused by a decreased of ferrochelatase activity and protoporphyrin accumulation in bone marrow erythroid cells. Patients develop skin lesions (erythema and edema) in the sun exposed areas, due to high concentration of protoporphyrin (Yoshida et al., 2018). Zebrafish *dracula* (*drc*) mutant exhibits a point mutation in the ferrochelatase gene, inducing accumulation of protoporphyrin which suggests that is a deficiency in the last enzyme involved in heme biosynthesis represented by ferrochelatase activity. *Dracula* mutants exhibit autofluorescent erythrocytes, light-dependent hemolysis, and liver malfunction, similar to conditions seen in humans (Childs et al., 2000). *Yquem* (*yqe^{tp61}*) is another zebrafish mutant developed for studying erythropoietic protoporphyria. *Yquem* mutant (*yqe^{tp61}*) has a loss-of-function mutation in uroporphyrinogen decarboxylase gene, another enzyme involved in the heme synthetic pathway (Wang et al., 1998).

Hereditary spherocytosis is a type of congenital hemolytic anemias resulting from plasma membrane protein deficiency leading to sphere-shaped red blood cells. Patients with hereditary spherocytosis have defects in the genes coding for red cell membrane proteins including ankyrin, spectrin, band-3 and protein 4.2 (Park et al., 2016). Zebrafish *riesling* (*ris*) carries a mutant β -spectrin gene and exhibits anemia due to erythrocyte hemolysis. Zebrafish β -spectrin shares 62.3% identity with the human ortholog (Liao et al., 2000).

Polycythemia vera (PV) is a myeloproliferative disease caused by clonal expansion of hematopoietic progenitors and it is characterized by erythrocytosis, often leukocytosis and/or thrombocytosis. PV patients have an increased risk of disease transformation to acute myeloid leukemia (AML) or to myelofibrosis (MF). In 2006 WHO (World Health Organization) declared this disease a myeloproliferative neoplasm (Devos et al., 2018). Zebrafish *jak2a^{V581F}* mutant, an ortholog of human *JAK2^{V617F}*, has various common features with polycythemia vera observed in human and can offer essential information about production mechanisms of disease. Expression of *jak2a^{V581F}* induced an increase in erythropoiesis and downregulation of erythropoietin

expression at both mRNA and protein level (Ma et al., 2009).

Fish models for leukocytes diseases

Acute lymphoblastic leukemia (ALL) is the most common type of pediatric cancer representing approximate 75% of all cases of leukemia which appear in childhood. The pathogenesis of ALL involves malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extramedullary sites. ALL can be separated into T-cell acute lymphoblastic leukemia (T-ALL) and B-cell acute lymphoblastic leukemia (B-ALL) (Konantz et al., 2019). The first zebrafish genetically engineered T-ALL model was established by expressing mouse c-Myc (Myc), or the chimeric enhanced green fluorescent protein (EGFP)-Myc fusion gene, driven by the lymphocyte-specific rag2 promoter (Langenau et al., 2003). In this system, the EGFP-labeled lymphocytes were tracked by fluorescence microscopy to detect neoplastic thymic expansion and infiltration into surrounding skeletal muscle and other organs, although involved in both T-ALL and B-ALL development in zebrafish (Garcia et al., 2018; Borga et al., 2019). All early rag2-driven ALL models developed in the 2000s exclusively induced T-cell neoplasia. (Jing and Zon, 2011). *Rag2-EGFP-mMyc* transgenic line was created to develop acute lymphoblastic leukemia, leukemic cells, expressing *scl* and *lmo2* orthologous genes (Chen et al., 2007). Because human *NOTCH1* gain-of-function mutations/deletions have been found in about 60% of T-ALL patients (Weng et al., 2004), being the most commonly mutated oncogene in this disease, Chen et al. (2007) generated transgenic zebrafish that express the intracellular domain of human NOTCH1 (ICN1) fused to EGFP under control of the rag2 promoter to develop an aggressive oligoclonal T-ALL.

Myeloid leukemia is another type of leukemia studied on *Danio rerio*. *AML1-ETO* translocation is the most common chromosomal translocation in acute myeloid leukemia. The ubiquitous expression of the AML1-ETO oncogene in *Danio rerio* embryos lead to premature lethality and requires an inducible expression through thermal shock promoter in transgenic fish (*hsp70:AML1-ETO*). *AML1-ETO* protein induced in embryos redirects erythroid progenitor cells to differentiate in granulocytes, a phenotype also seen in human acute myeloid leukemia (Jing and Zon, 2011). TEL-JAK2 fusions have been identified in patients with lymphoblastic and myeloid leukemia that result in constitutive activation of the JAK2 kinase domain. To mimic the clinical situation, *tel-jak2a* fusion oncogene was expressed in developing zebrafish embryos under control of the *sp1* promoter, which is strongly expressed in myeloid precursor cells. In embryos, *tel-jak2a* expression leads to disruption of normal embryonic hematopoiesis, including perturbation of the myeloid and erythroid lineages (Onnebo et al., 2005).

Leukocyte adhesion deficiency (LAD) are autosomal recessive disorders, characterized by the inability of neutrophils to emigrate from the circulation to the sites of injury, because of dysfunctional selectin- or integrin-mediated adhesion events, resulting in recurrent bacterial infections. Zebrafish expressing the dominant inhibitory *Rac2-D57-N* mutation shows defects in neutrophil cell polarization and migration, increase in neutrophil mobilization from hematopoietic tissue and a defect in egress from the vasculature to sites of tissue damage, resulting in neutrophilia (Deng et al., 2011).

Congenital neutropenia comprises a group of genetic diseases characterized through decrease in the absolute number of neutrophils in circulation. Some diseases are limited to hematological deficiency, while other are associated with pancreas, brain, heart, skeletal system and skin diseases (Bellanné-Chantelot et al., 2018). Granulocyte colony-stimulating factor receptor (G-CSFR), encoded by the *CSF3R* gene, plays a major role in the production and function of neutrophils in mammals, *CSF3R* mutations leading to neutropenia in humans. Zebrafish G-CSFR is encoded by the *csf3r* gene orthologue, which has been shown to have a conserved role in developmental myelopoiesis.

Zebrafish *csf3r* mutants with significantly decreased numbers of neutrophils from embryonic to adult stages, did not respond to Granulocyte colony-stimulating factor (G-CSF), and displayed enhanced susceptibility to bacterial infection (Pazhakh et al., 2017; Basheer et al., 2019).

WHIM (Warts, Hypogammaglobulinemia, Infections, Myelokathexis) syndrome is a congenital immune deficiency characterized by hypogammaglobulinemia, infections, myelokathexis (retention of neutrophils in the bone marrow), leukopenia, human papillomavirus (HPV)-induces warts, reduced long-term immunoglobulin G (IgG) titers and severe chronic neutropenia (Kawai and Malech, 2009; Rissone and Burgess, 2018). WHIM syndrome is caused by a gain in function mutation in CXCR4 (CXC chemokine receptor 4) that induce the truncation of its carboxy-terminal domain (Rissone and Burgess, 2018). *Danio rerio* was used for studying this disease because it allows visualization of the neutrophils circulation due to transparency. The zebrafish model showed retention in hematopoietic tissue and an impairment of neutrophils recruitment and motility to wounds and inflamed tissue, similar to human (Walters et al., 2010).

Table 4 Human hematological diseases studied on *Danio rerio*

| Hematological disease | Mutant | Authors |
|--|-----------------------------------|---|
| Congenital dyserythropoietic anemia | <i>Retsina (ret)</i> | Paw et al., 2003 |
| Severe hemolytic congenital anemia | <i>Merlot(mot)/Chablis (cha)</i> | Shafizadeh et al., 2002 |
| Diamond-Blackfan anemia | <i>rpl11</i> | Danilova et al., 2011; Chakraborty et al., 2018 |
| | <i>rps29</i> | Taylor et al., 2012 |
| | <i>rps24</i> | Song et al., 2014 |
| | <i>rpl35a</i> | Yadav et al., 2014 |
| | <i>rps27</i> | Wang et al., 2015 |
| | <i>rps14</i> | Narla et al., 2014; Ear et al., 2016 |
| | <i>rps7</i> | Antunes et al., 2015 |
| | <i>rpl5</i> | Wan et al., 2016 |
| Fanconi anemia | <i>fancd2</i> | Liu et al., 2003 |
| | <i>rad51</i> | Bothof et al., 2017 |
| Microcytic anemia | <i>Chardonnay (cdy)</i> | Donovan et al., 2002 |
| | <i>Zinfandel (zin)</i> | Brownlie et al., 2003 |
| | <i>Chianti (cia)</i> | Wingert et al., 2004 |
| Sideroblastic anemia | <i>Sauternes (sau)</i> | Brownlie et al., 1998 |
| | <i>Weissherbst (weh)</i> | Donovan et al., 2000 |
| Erythropoietic porphyria | <i>Yquem (yqe^{tp61})</i> | Wang et al., 1998 |
| | <i>Dracula (drc)</i> | Childs et al., 2000 |
| Hereditary spherocytosis | <i>Riesling (ris)</i> | Liao et al., 2000 |
| LAD | <i>Rac2</i> | Deng et al., 2011 |
| Acute myeloid leukemia | <i>tel-jak2a</i> | Onnebo et al., 2005; 2012 |
| | <i>stat5.1</i> | Lewis et al., 2006 |
| | <i>Hsp70-Cre</i> | Feng et al., 2007 |

| | | |
|--|---|---|
| | <i>kRAS^{G12D}</i> | Le et al., 2007 |
| | <i>Tg (hsp:AML1-ETO)</i> | Yeh et al., 2008 |
| | <i>zspi1-MYST3/NCOA2</i> | Zhuravleva et al., 2008 |
| | <i>CG1-strain</i> | Smith et al., 2010 |
| | <i>NUP98-HOXA9</i> | Forrester et al., 2011; Deveau et al., 2015 |
| | <i>RUNX1-ETO (AML1-ETO)</i> | Cunningham et al., 2012 |
| | <i>hRAS^{V12G}</i> | Alghisi et al., 2013 |
| | <i>MYCN: HSE:EGP</i> | Shen et al., 2013 |
| | <i>sp1:FLT3-ITD-2A-EGFP</i> | Lu et al., 2016 |
| Acute lymphoblastic leukemia | <i>Rag2-EGFP-mMyc</i> | Langenau et al., 2003 |
| | <i>Rag2-NOTCHCD-EGFP</i> | Chen et al., 2007 |
| | <i>Efl alpha: EGFP-TEL-AML1</i> | Sabaawy et al., 2006 |
| | <i>Rag2-GFP</i> | Mizgirev and Revskoy 2010 |
| | <i>Hsp70-Cre</i> | Feng et al., 2010 |
| | <i>Rag2-MYC-ER</i> | Gutierrez et al., 2011 |
| | <i>rag2-Myc-Notch^{ICD}</i> | Blackburn et al., 2012 |
| | <i>lck:EGFP</i> | Ridges et al., 2012 |
| | <i>rag2:MYC-ER; rag2:EGFP; rag2:EGFP-bcl2</i> | Reynolds et al., 2014 |
| Severe congenital neutropenia | <i>csf3r</i> | Pazhakh et al., 2017 |
| WHIM syndrome | <i>WHIM</i> | Walters et al., 2010 |
| Congenital amegakaryocytic thrombocytopenia | <i>mpl</i> | Lin et al., 2016 |
| Wiskott-Aldrich syndrome | <i>wasp</i> | Cvejic et al., 2008 |
| Vera polycythemia | <i>Jak2a^{V581F}</i> | Ma et al., 2009 |
| Myelodysplastic syndrome | <i>crimsonless (crs)/hspa9b</i> | Craven et al., 2005 |
| | <i>prpf8/cph (cephalophõnus)</i> | Keightley et al., 2013 |
| | <i>pu.1^{G242G}/spi1</i> | Sun et al., 2013 |
| | <i>Tet2</i> | Gjini et al., 2015 |
| | <i>sf3b1</i> | De La Garza et al., 2016 |
| | <i>c-myb^{hyper}</i> | Liu et al., 2017 |
| | <i>asxl1</i> | Gjini et al., 2019 |

Wiskott-Aldrich syndrome (WAS) is a rare X-linked primary immunodeficiency disorder characterized through microthrombocytopenia, recurrent infections, eczema, bloody diathesis and a high frequency of autoimmunity and malignancy (Rissone and Burgess, 2018; Mansour et al., 2020). This disorder is caused by mutations in the *WAS* gene which encodes WAS protein (WASp). WASp is a 502-amino acid protein expressed in cytoplasm and nucleus of hematopoietic cells and involved in signal transduction cell motility, endocytosis and phagocytosis (Byrne et al., 2018). Cvejic et al. (2008) used zebrafish embryos and larvae to observe the effects of morpholino knockdown of WASP1 on leukocytes migrations in response to a wound. After three days post-fertilization they observed a reduction in number of recruited neutrophils and macrophages from wounds. Also, knockdown of zebrafish WASps not alter the total number or developmental dispersal of leukocytes in the organism, but disrupts the wound inflammatory response by perturbing the mechanism by which these cells are

oriented towards affected tissue (Cvejic et al., 2008). A null mutant of zebrafish WASp shows defects in immune-cell-mediated resistance to bacterial infection and wound-induced inflammatory response. These defects mimic the symptoms of human WAS patients, as defects in immune-cell-mediated resistance to bacterial infection (Jones et al., 2013).

Fish models for thrombocytes diseases

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare inherited autosomal recessive disease characterized by severely low number of megakaryocytes and thrombocytopenia. This disorder appear in childhood and it is often discovered in the first day of life at least within the first month (Al-Qahtani, 2010). The cause is a mutation in the human myeloproliferative leukemia protein gene (MPL) (Al-Qahtani, 2010; Lin et al., 2016). Lin et al. (2016) used transcription activator-like effector nuclease (TALEN) technology to create zebrafish *mpl*

mutant for studying human CAMT. They discovered that disruption of zebrafish *mpl* led to an important reduction in platelets and a high bleeding tendency. Also, *mpl*-deficient zebrafish replicate human thrombocytopenic phenotypes through impairment of evolutionarily conserved TOP/MPL/JAK pathway (Lin et al., 2016).

Fish models for other hematological diseases

Myelodysplastic syndrome (MDS) comprises a heterogeneous group of hematological disease characterized by ineffective hematopoiesis due to dysplasia and accelerated apoptosis of hematopoietic progenitors and their descendants. MDS is associated with many diseases, as anemia, erythroid dysplasia, cytopenia or acute myeloid leukemia (Craven et al., 2005). MDS is caused by a mutation in TET2 (Tet methylcytosine dioxygenase 2p) which is one of the most frequently mutated tumor suppressor genes in this disease (Langemeijer et al., 2009). Zebrafish *crimsonless* mutants are anemic and display defective blood cell differentiation followed by apoptosis and a decrease in erythrocytes, granulocytes and progenitor hematopoietic cell number. *Crimsonless* mutants appeared visibly normal until 33 hours post-fertilization, when was observed hypochromic blood, despite the normal number of cells in circulation (Craven et al., 2005). Also, Gjini et al. (2015) established zebrafish lines with loss of function mutations in *tet2* (ten-eleven translocation 2). *Tet2* gene encodes a member of the TET family of DNA methylcytosine oxidases involved in conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) to initiate the demethylation of DNA within genomic CpG islands. Zebrafish *Tet2* mutant is characterized by an increased number of progenitor and myelomonocytic cell and a decrease in erythrocytes within kidney (Gjini et al., 2015).

Conclusions

Even if fish are the most numerous and diverse group of all vertebrates and hematological characterization is difficult, sometimes impossible, study of blood cells in different fish species gives us important comparisons between fish and other vertebrates, including human, highlighting aspect of evolution. Considering all the similarities, but also differences between fish and human blood cells, fish, especially *Danio rerio* are considered model organisms for discovering molecular mechanisms underlying human hematological disease and for finding new therapeutic methods that can be applied to humans. However, there are controversies regarding the use of *Danio rerio* as a model organism due to large evolutionary distance from human. Some researchers claim that the results obtained on this species often require further validation using evolutionarily closer model organisms to human. Many similarities can be

observed between fish and human blood cells and following comparisons between their hematopoiesis and lymphopoiesis has been proven that genes that underlie blood cells development are highly preserved throughout evolution. Furthermore, *ikaros*, *rag-1*, *rag-2* and *lck* genes that are found in human were observed in *Danio rerio*, this fact revealing the spatio-temporal proximity between groups of vertebrates, thus, studies realized on fish can offer essential information about molecular mechanism which causes the appearance of various human hematological diseases and the discovery of possible therapeutic methods. Therefore, *Danio rerio* and the other fish species can be important model organisms which can be used in studying human hematological disease, as anemia, leukemia, LAD, neutropenia, polycythemia, porphyria, spherocytosis, myelodysplastic syndrome, WHIM syndrome, Wiskott-Aldrich syndrome and thrombocytopenia.

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