INTRODUCTION

- Three types of PNH have been described:
  a) florid PNH;
  b) PNH in the context of bone marrow failure (BMF);
  c) subclinical PNH, in the context of BMF.
- In any case, Flow Cytometry (FCM) plays a crucial role in detecting and monitoring PNH clones. In BMF syndromes, as Aplastic Anemia (AA), Unexplained Cytopena (UC) and MDS, small PNH clones can be detected, so that high-sensitivity FCM is requested.
- In 2010, an Italian archive of FCM-detected PNH clones was created on a multilaboratory basis. Ninety-eight laboratories participated in the project, and just 68 (59.4%) has been active.
- The aim was twofold: a) to provide a large dictionary of Italian PNH clones; b) based upon stringent rules regarding the compilation of records, to obtain an auto-educational effect on participating laboratories.
- Here, we describe composition and evolution of PNH clones as assessed by analyzing data from the ClonoPNH Archive (http://www.clonepnh.com).

METHODS

- We analyzed data from ClonoPNH Archive to evaluate:
  a. how many PNH clones have been identified so far and how they are classifiable on the basis of the Reason for Testing (RFT) reported in clinical request;
  b. how many clones received more than one FCM determinations;
  c. among these, which was the initial RFT as well as the fate of the clone.
- Data refer to analysis registered in ClonoPNH Archive up to 24/05/2013.

RESULTS

- We collected 2619 records, including negative, positive and follow-up analyses.
- 392 PNH clones were identified since 2001 and recorded into the Archive since 2010. In 38 cases (9.7%) the main PNH clone (PNH5) was accompanied by a PNH2 component, of minor size.
- The 392 PNH clones were categorized into 11 classes, according to their size (Fig. 1). This distribution is similar to that reported by Mavola et al (2011).
- Among PNH3 clones, Haemoglobinuria was the most frequent condition bringing to FCM analysis (43.4% of cases), followed by Aplastic Anemia (AA, 18.1%), MDS (11.7%), other (9.9%), Idiopathic Cytopena (IC, 7.4%), Hemolytic Anemia (HA, 5.9%), Bone Marrow Failure (BMF, 5.1%), Atypical Venous Thrombosis (AVT, 3.6%) (Fig. 2).
- The 392 clones were categorized into 3 classes according to their size, determined as the percentage PNH cells in peripheral granulocytes: 0.01-10% (158 clones, or 44%), defined as "small", 10.1-70% (82 clones, or 19.6%, defined as "intermediate"), 70-100% (138 clones, or 32.9%, defined as "large"). In 2.6% of cases, it was not possible to categorize PNH3 clones.
- The distribution of each category depending on the RFT was analyzed too (Fig. 3). In case of BMF and MDS, the smallest clones have the highest percentage of analysis.
- Reagent choice significantly changed, leading to a stable FCM protocol consisting of FLAER and CD24 for granulocytes, FLAER and CD14 for monocytes, CD59 for erythrocytes (Fig. 4).
- Thanks to the follow-up visits, it is possible to obtain a more concrete view of the clone size over time. The possibility to relate the clone trend with the bone marrow activity allows to create a very important synergy between cytometry and clinicians, and guarantees the regular monitoring of patients, thanks to their close collaboration.
- Among the 392 PNH3 clones, 107 received more than one FCM test. Twenty-nine of them (28.9%) showed a change in category (17 increased and 14 decreased). Just 4 clones jumped from “smallest” to “biggest” category or vice versa (2 increased and 2 decreased) (Table 1).
- One of the major innovations introduced in the ClonoPNH Archive regards the possibility to enter the data relating the blood cell count, as absolute count, with the opportunity to establish a relation with the bone marrow activity (Fig. 5).

CONCLUSIONS

This is the first multilaboratory relational database of FCM-detected PNH clones described so far. An auto-educational role was reached, since general sensitivity and the number of identified clones increased progressively (Fig. 6) and reagent choice significantly changed, leading to a stable FCM protocol (Fig. 4). The identified PNH3 clones were analyzed. Most of clones showed a very big (90.1-100%) or a small (0.01-10%) size. 107 clones were studied with more than one determination, and most of them had no change in category. 31 clones showed some change in category (17 increased and 14 decreased), but the migration between “big” and “small” category was rare (4 cases), suggesting that these two categories are sustained by different backgrounds and pressures.

Table 1. Cases with 1 or 2 categories' changes

<table>
<thead>
<tr>
<th>Type of change</th>
<th>N. of cases</th>
<th>%</th>
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<tbody>
<tr>
<td>Increase of 1 category</td>
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<td>14.0%</td>
</tr>
<tr>
<td>Decrease of 1 category</td>
<td>12</td>
<td>11.2%</td>
</tr>
<tr>
<td>Increase of 2 categories</td>
<td>2</td>
<td>1.9%</td>
</tr>
<tr>
<td>Decrease of 2 categories</td>
<td>2</td>
<td>1.9%</td>
</tr>
<tr>
<td>No changes</td>
<td>68</td>
<td>63.6%</td>
</tr>
<tr>
<td>Not valuable</td>
<td>8</td>
<td>7.5%</td>
</tr>
</tbody>
</table>

Fig. 1. Distribution of PNH clone size in the 392 PNH clones

Fig. 2. Distribution of RFTs in the 392 PNH clones

Fig. 3. Distribution of categories depending on the RFT

Fig. 4. Reagent choice in the years

Fig. 5. Blood cell count

Fig. 6. Analysis with identified clones in the years


For further details, please contact dr. Elisa Cannizzo, e-mail: elisacannizzorelisa@gmail.com – Emmeidi Srl div Solaris, e-mail: solarsolarscoro.com