Pneumocystis jirovecii infections in (non HIV-infected) hematology patients: *Part A: Biological aspects*

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Pneumocystis infections pathophysiology

- *Pneumocystis jirovecii*: specific to humans
- Transmission by airborne route
- Acquisition in humans most likely by person-to-person spread (Thomas CT et al. NEJM 2004; 24: 2487)
- Nearly universal seropositivity in immunocompetent children 2 years of age (Vargas SL et al. Clin Infect Dis 2001; 32: 855)
- Primary infection is generally an asymptomatic or mild, self-limiting upper respiratory tract infection
- Responsible for *Pneumocystis jirovecii* pneumonia (PjP) in immucompromised patients

Recommendation for stainings

- We recommend the use of immunofluorescent staining as the most sensitive microscopical method for detection of *Pneumocystis* **(AII)**

- Considerations:

  - Other stains can be used to detect cysts [optical brighteners (such as Calcofluor White and Blankophor), Toluidine Blue O, methenamine silver] or trophic forms (Giemsa). Considering the inconsistency of studies comparing these stainings and the absence of clinical consequences of detection of the 2 forms of the fungus, no recommendation for the specific diagnosis of PjP is made.

  - Whichever stain is used, it should be remembered that the sensitivity and specificity of direct microscopy is dependent on the quality, quantity and type of the specimen, the quality of the microscope and the expertise and mycological experience of the operator.

Recommendation for samples

- We recommend the use of BAL fluid as it is the most sensitive specimen with good negative predictive value **(AII)**

- If BAL is not an option then, we recommend to use other suitable respiratory specimens [induced sputum (IS), adequate upper respiratory specimens (URS)] as alternative **(BII)**

PCR method in respiratory samples for PjP diagnosis

- **Real-time PCR** is the only PCR method that meets the MIQE [1] recommendations for diagnosis **(AII)**
- Express the results as **quantitative data** (equivalent fungus/mL, Cq or preferably copy/mL)
- Consensual quantitative **threshold** (qPCR) corresponding to positive immunofluorescence is still not defined
- **Probes** are preferable to intercalating dyes [2]
- Use **dedicated rooms** (Mix preparation/Extraction/amplification)
- Use **enzymatic prevention** of amplicons contamination (UNG) [2, 3]
- Use **internal control** for DNA extraction and DNA amplification with foreign DNA (avoid human DNA or DNA from human virus) [1, 2, 4]
- Use **external quality control** [5]

(1) Bustin, S.A. et al. *Clin Chem* (2009)
(2) Bretagne, S. *Curr Infect Dis Rep* (2011)
(3) Burkardt, H.J. *Clin Chem Lab Med* (2000)
(4) Hoorfar, J. et al. *J Clin Microbiol* (2003)
(5) Linssen CMF et al. *J Med Microbiol* (2006)

Recommendation for other diagnostic test

- We recommend the use of BD-glucan in serum as a laboratory diagnostic tool for the diagnosis of PjP **(AII)**

- We do not recommend the use of BD-glucan in serum for the follow-up of PjP treatment **(AII)**

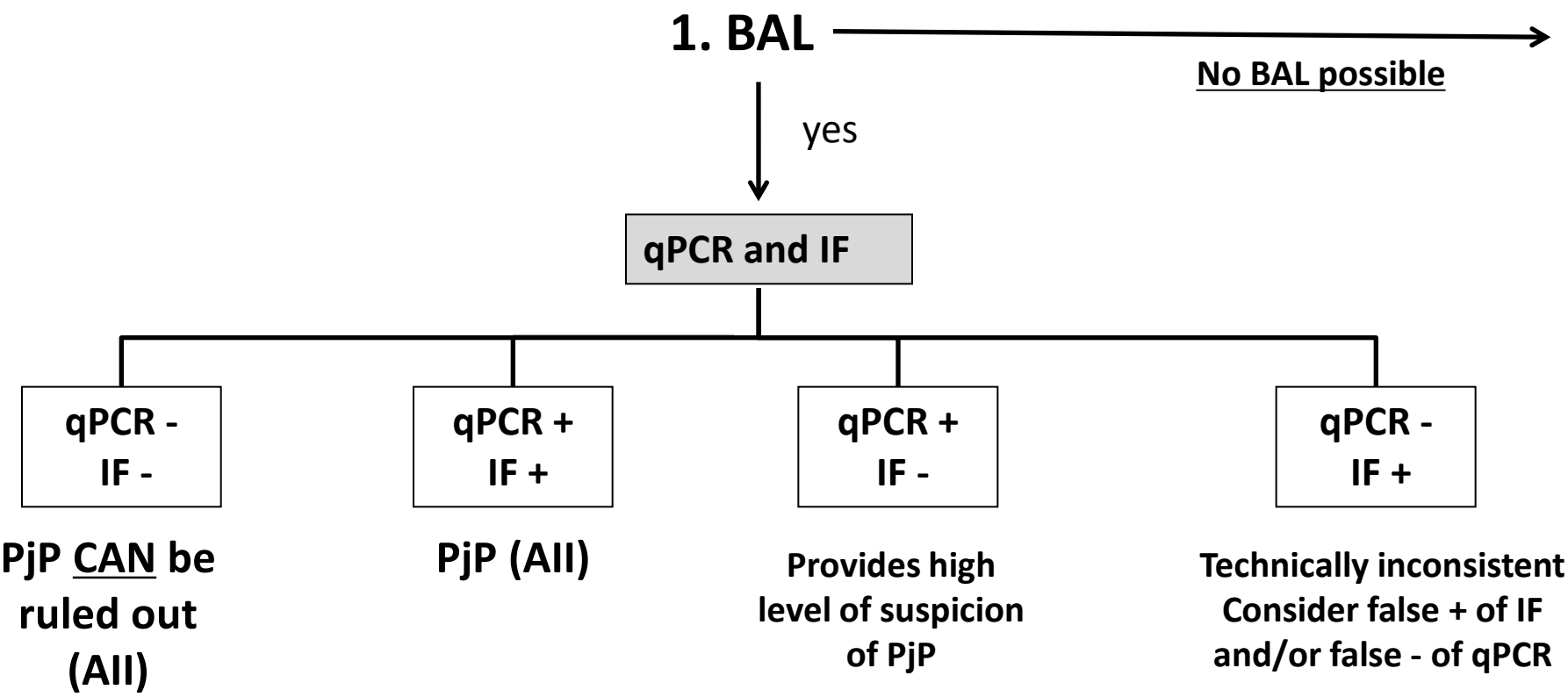
    - Considerations:

      Negative results can rule out the diagnosis of PjP

      Positive results may suggest alternative fungal infections and should trigger additional diagnostic work-up

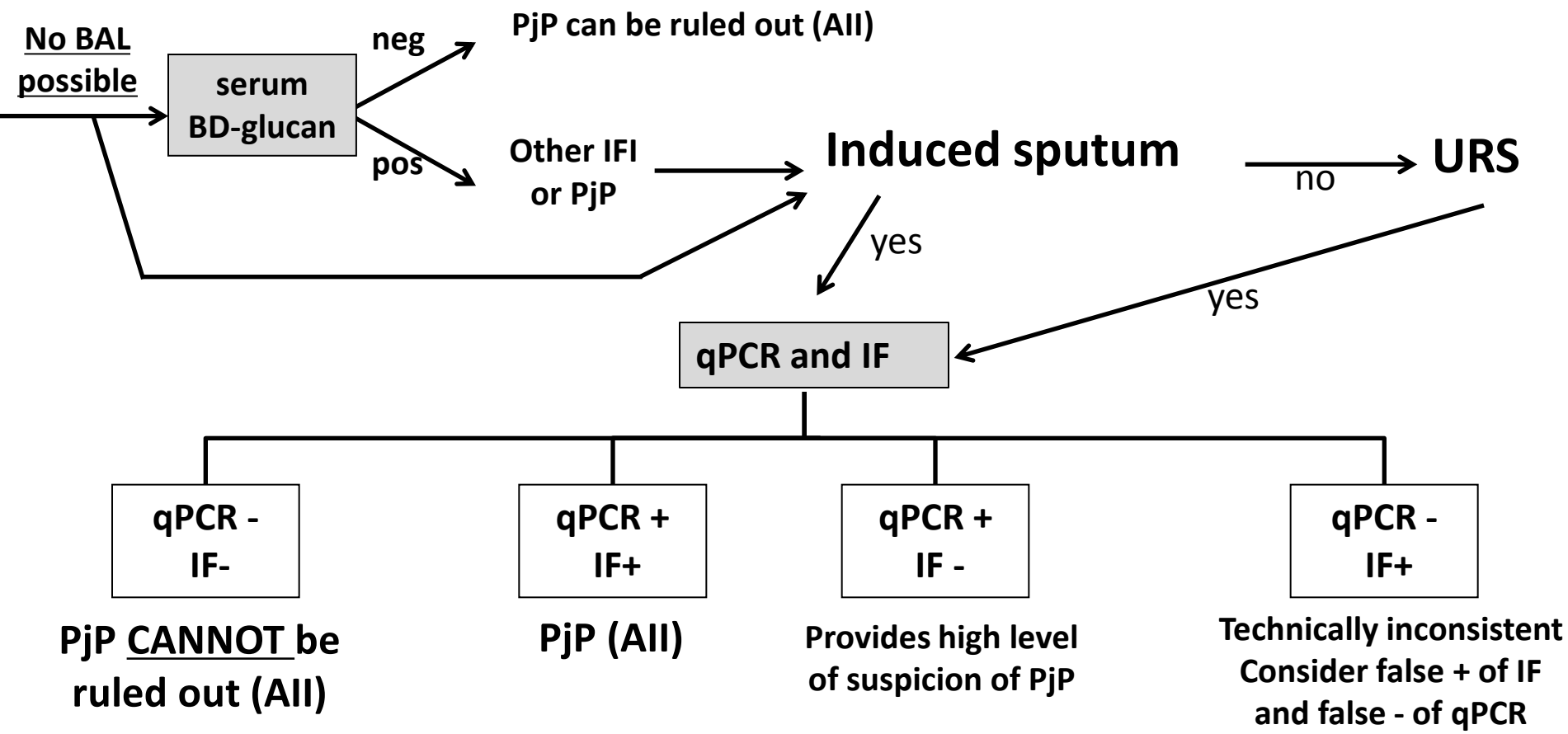
      Absence of data for supporting BD-glucan in BAL fluid

Organization chart for the diagnosis of PjP



Considerations :

- A high fungal load is suggestive of PjP, although thresholds are not definitively defined
-For low fungal loads consider BD-glucan in serum to support the diagnosis



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Other considerations

(1) Quantification of qPCR results can be impacted by delay (> 3days) in BAL procedure if patient is given cotrimoxazole treatment

(2) When immunofluorescence is performed before qPCR and is positive, some laboratories do not perform additional tests (qPCR, BD-glucan)

(3) Some laboratories have already validated their own qPCR thresholds corresponding to positive IF, and do not perform microscopic examination anymore.

Recommendation for additional molecular tests

Outbreaks

- We recommend genotyping using MLST (cytb, mito26SrRNA, sod) to investigate suspected outbreaks **(AII)**
    - Considerations:

      Seek expert laboratory assistance

Treatment failure

- We do not recommend the routine detection of DHPS mutations in case of treatment failure **(BII)**
    - Considerations:

      DHPS mutations do not affect response to high dose cotrimoxazole