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CCMG Practice Guidelines for Cytogenetic Analysis

C. Recommendations for the indications, analysis and reporting of cancer specimens

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These guidelines are compiled from various sources, and are based primarily on expert opinion and descriptive papers that demonstrate the value of cytogenetics in the diagnosis, prognosis and management of neoplasia. The indications for cancer cytogenetics are not all-inclusive, and may be extended where there is local interest. These guidelines are minimum recommendations only. They are subject to the discretion of the laboratory director and the requirements and capabilities of the local laboratory. It should be recognized that these guidelines are based on information that is available at the present time, and will change with advances in cytogenetic knowledge.

Standard of practice i.e., in house protocols should be established for the processing of tissue and cell types, culture setup, analysis, quality control and turn-around time based on the reason for cytogenetic testing (e.g., initial diagnosis versus follow-up) and the clinical utility of the cytogenetic information (e.g., diagnosis/prognostic value/selection of therapy).

All cytogenetic findings should be interpreted in the context of clinical, morphological and other laboratory findings, whenever possible.

C.1 Indications for Cancer Cytogenetic Investigations

A complete cytogenetic analysis of bone marrow should be performed at the time of initial evaluation to establish the cytogenetic profile, and at intervals thereafter, as clinically indicated, to detect persistence of an abnormal clone or evidence of genetic/clonal evolution. A standard of practice for the cancer cytogenetics of specific neoplastic disorders should be established at each genetic centre.

The following neoplastic disorders are listed according to the 2008 World Health Organization (WHO) (Classification of Tumours of Haematopoietic and Lymphoid Tissues. 2008. eds. Swerdlow et al. International Agency for Research and Cancer, Lyon, France).

a) Chronic Myeloproliferative Neoplasms (MPN)

1) Chronic myelogenous leukaemia (CML):
   Karyotype at diagnosis; FISH (BCR/ABL1) as required to confirm diagnosis in unusual cases, or cases with no/few metaphases (or to establish the signal patterns, detect cryptic rearrangements, etc). Follow-up investigation may be indicated for staging purposes or to monitor the effect of treatment.

   Karyotype or FISH may be indicated at diagnosis for the following disorders:

2) Polycythaemia vera (PV)

3) Chronic idiopathic myelofibrosis (CIMF)

4) Essential thrombocythaemia (ET)

5) Chronic eosinophilic leukemia (CEL)

6) Chronic neutrophilic leukemia (CNL)

b) Myeloid and lymphoid neoplasms associated with eosinophilia

At diagnosis, karyotype and/or FISH to detect abnormalities of PDGFRB (5q31~q33) or FGFR1 (8p11) and FISH to detect cryptic abnormalities of PDGFRA (4q12).

c) Myelodysplastic/Myeloproliferative neoplasms

1) Chronic myelomonocytic leukemia (CMML):
   Karyotype and/or FISH for BCR/ABL1 to rule out t(9;22) and PDGFRB (5q31~35); and FISH for PDGFRA (4q12) (if eosinophilia is present).

2) Atypical chronic myeloid leukemia (aCML):
   Karyotype and/or FISH for BCR/ABL1 to rule out t(9;22).

3) Juvenile myelomonocytic leukemia (JMML):
   Karyotype at diagnosis.

d) Myelodysplastic syndromes

Karyotype at diagnosis, especially in the BMT-eligible patient. Follow-up investigation may be indicated at disease progression and after treatment.

e) Acute leukemia (Myeloid and Lymphoid)

Karyotype all cases. FISH as indicated based on chromosome morphology, clinical and pathological features. If an abnormality is present, follow-up after treatment or at relapse may be indicated. If an abnormal clone is not detected, re-investigation at relapse may be indicated in a second attempt to detect a disease-related clone.

f) Mature B cell neoplasms

1) Chronic Lymphocytic Leukemia (CLL):
   FISH to detect abnormalities such as: +12, del(13)(q14), or deletion of ATM and p53 can be performed. The specific FISH loci to be tested and need for conventional karyotype would depend on the policy of the local centre.

2) Plasma cell myeloma:
   At a minimum, FISH to detect abnormalities such as t(4:14) and deletion of p53 should be performed. The specific FISH loci to be tested and need for conventional karyotype would depend on the policy of the local centre. Investigations should preferentially target the analysis of plasma cells.

Karyotype and/or FISH may be appropriate at diagnosis in selected cases, in consultation with the pathologist/clinician, for the following disorders:

3) Marginal Zone Lymphoma (MZL)/MALT lymphoma

4) Follicular Lymphoma (FL)

5) Mantle Cell Lymphoma (MCL)

6) Diffuse Large B-Cell Lymphoma (DLBCL),

7) Burkitt Lymphoma (BL)

g) T-cell Neoplasms

Karyotype and/or FISH may be appropriate at diagnosis in selected cases, in consultation with the pathologist/clinician for the following disorders:

1) T-cell Prolymphocytic Leukaemia (T-PLL)

2) Hepatosplenic T-cell Lymphoma

3) Anaplastic Large Cell Lymphoma (ALCL)

h) Solid tumors

Karyotype and/or FISH may be appropriate at diagnosis for small round cell tumors of childhood, selected sarcomas, lipomatous tumors, and other tumors in consultation with the pathologist/clinician. In consultation with the pathologist/clinician., FISH may also be used for the diagnosis and prognosis of some carcinomas, e.g. bladder, prostate, breast.

C.2 Recommendations for the Processing of Cancer Cytogenetic Specimens

Specimens include bone marrow, blood, lymph node, solid tumor, needle aspirates, fluids and effusions. There are many methods for culturing and harvesting cancer specimens. Some recommendations, based in part on the ACMG Standards and Guidelines 2006, are as follows.

1) Bone marrow is the tissue specimen of choice for the analysis of suspected hematologic disorders including aplastic anemia and chronic myeloproliferative disorders. When bone marrow is not available, unstimulated peripheral blood may provide useful information if sufficient immature cells are present. Culture conditions should be optimized for the specific hematologic disorder suspected. A bone core biopsy may sometimes be the only option. A bone core biopsy should be mechanically or enzymatically minced to yield cell suspensions, which are then cultured as for bone marrow aspirates.

2) Cultured (overnight or short term) bone marrow harvests are preferred for analysis. A direct harvest may also provide metaphases for analysis. For acute leukemias, unstimulated short-term cultures are recommended. If sufficient specimen is received, at least two cultures should be initiated, one of which should be a 24-hour *or* overnight culture.

3) Blood specimens produce better results when they are harvested after short term to 48 hours, rather than by direct harvest.

4) In most cases, it is preferable to analyze unstimulated bone marrow or blood specimens. B-cell or T-cell mitogens for mature B- and T- cell disorders and other culture additives such as hormones and growth factors may be added to the culture medium to encourage clonal divisions in specific disorders. However, unstimulated cultures should always be examined in these cases as additives may mask an abnormal clone.

   Mature B-cell neoplasms (e.g. CLL; plasma cell myeloma): Although there is no consensus on this point, some laboratories have had success in identifying abnormal clones with the addition of B-cell mitogens.

   Mature T-cell neoplasms (e.g. T-cell leukemia/lymphoma): T-cell mitogens may be helpful.

5) Lymph node is the tissue of choice for the analysis of suspected lymphoma. Lymph nodes should be disaggregated mechanically and/or enzymatically to create a single cell suspension, then cultured and harvested as for bone marrow specimens.

6) Effusions and fluids are harvested after a direct or 24-hour culture.

7) Solid tumour tissue should be disaggregated mechanically and/or enzymatically to create a suspension of single cells and small cell clusters. Whenever possible, solid tumors should be harvested within one week of establishing the culture. Tumors that have been cultured for longer periods may be overgrown by normal fibroblasts. Normal results should be interpreted with caution, with a statement including length of time in tissue culture.

8) The failure rate for bone marrows and neoplastic blood specimens should not exceed 10%. Failures include both culture failures and inadequate specimens.

C.3 Recommendation for Chromosome Analysis of Cancer Specimens

a) General

1) Consultation with the clinician and/or pathologist is recommended to assist in the analysis and interpretation of cancer specimens whenever possible. The results should be correlated with other laboratory and clinical findings.

2) Cytogenetic follow-up may be indicated in specific circumstances and at appropriate intervals to evaluate disease progression or the effect of treatment.

3) The following are recommended minimum chromosome analysis guidelines. The extent and focus of the analysis will vary with the clinical situation. FISH or molecular methods may replace or supplement chromosome analysis in some situations. It is recommended that laboratories develop local or regional testing algorithms, based on the availability of FISH, molecular diagnostics, and other laboratory tests

4) The numbers of cells to be analyzed at diagnosis are minimum numbers, which are supported by the descriptive studies of Swansbury (1998) and Kuffel *et al* (1991). As described by Swansbury (1998), some disorders more frequently have a low percentage of abnormal cells. For these diagnoses, analysis of more cells may be warranted. In addition, if the presence of normal cells or clonal evolution is significant, more cells may have to be examined or analyzed.

5) For chromosome analysis, metaphases that are selected should represent the range of chromosome morphology on the slides, i.e. select metaphases with poor chromosome morphology as well as those with good morphology.

6) A normal cytogenetic result at diagnosis generally does not warrant further cytogenetic analysis following treatment and remission. It should be noted, however, that cytogenetic analysis may sometimes be requested to rule out a therapy related abnormality (e.g. MDS), rather than disease recurrence.

7) For determination of engraftment status, molecular methods for determination of recipient versus donor cells are the preferred methodology. For determination of relapse, cytogenetic G-band analysis and/or interphase FISH analysis may be warranted, dependant on the proportion of donor to recipient cells and in consultation with the pathologist/clinician.

b) Chronic Myelogenous Leukemia and other Chronic Myeloproliferative Diseases

<u>At diagnosis</u>: Examine sufficient metaphases (a minimum of 10) to confirm the presence of an abnormal clone at diagnosis, and for pre-transplant assessment. When disease transformation is suspected, examine additional metaphases to rule out secondary abnormalities. Analyze at least 20 metaphases if normal.

<u>Post treatment monitoring:</u>
Examine sufficient metaphases (a minimum of 10) to confirm the presence of the original abnormal clone. If normal, examine at least 25 metaphases.

Karyotype at least one metaphase per stemline and significant sideline. A normal metaphase (when present) should be printed; a normal karyotype is recommended.

c) Acute Leukemia and Myelodysplasia

At diagnosis: Analyze enough metaphases (a minimum of 10) to confirm the presence of an abnormal clone (as per ISCN 2009). If only normal metaphases are found, or if the presence of clonal evolution is significant, analyze or examine at least 20 metaphases. Exception: In cases of confirmed pediatric pre B cell ALL, identification of the abnormal clone by more extensive analysis is warranted.

Post treatment monitoring:
Examine sufficient metaphases (a minimum of 10) to confirm the presence of the original abnormal clone. If normal, examine at least 25 metaphases.

Karyotype at least one metaphase per stemline and significant sideline. A normal metaphase (when present) should be printed; a normal karyotype is recommended.

d) Mature B cell neoplasms, Malignant Lymphoma, and Solid Tumors

At diagnosis: Analyze sufficient metaphases (a minimum of 10) to identify an abnormal clone, or 20 metaphases if normal.

Post treatment monitoring:
Examine sufficient metaphases (a minimum of 10) to confirm the presence of the original abnormal clone. If normal, examine at least 25 metaphases.

Karyotype at least one metaphase per stemline and significant sideline. A normal metaphase (when present) should be printed; a normal karyotype is recommended.

C.4 Recommendations for FISH Analysis of Cancer Specimens

a) General

1) In some situations, FISH is helpful in patient management because it provides greater sensitivity and/or a more rapid result than chromosome analysis. The extent of analysis will depend on local resources and the availability of molecular diagnostics. The guidelines below are for FISH applications that have been demonstrated to be of clinical value (For details, refer to ACMG Standards and Guidelines, 2nd Edition 2006).

2) FISH on interphase nuclei can be used for chromosome enumeration and for some types of rearrangement detection. Metaphase FISH can assist in the identification of markers and unusual or variant chromosome rearrangements.

3) FISH can be performed on any source of fresh, frozen, fixed and paraffin embedded tissue or cell including touch preparations and cytology slides.

4) All probes should be validated and cut-off values determined in-house, before clinical use (Refer to ACMG Standards and Guidelines, 2nd Edition 2006).

5) The limitations of FISH analysis must be stated in the report, when appropriate.

6) For the detection of translocations in interphase nuclei, probe sets that result in an extra signal with a single fusion or double fusions should be used, whenever possible.
7) FISH signals should, in general, be scored as instructed by the manufacturer of the probe/kit.

8) All interphase FISH analyses should be performed by at least two qualified individuals. If there are discrepant results, additional nuclei may be examined by a third technologist.

b) Sex Chromosomes

After bone marrow transplantation with an opposite-sex donor, examine a minimum of 200 interphase nuclei or 50 metaphase cells for the X and Y chromosome, using dual-color probes (Dewald *et al.*, 1993, 1998).

c) Numerical Abnormalities

1) At diagnosis, FISH using centromeric probes can be used to examine poor quality metaphases or interphase nuclei for numerical chromosome abnormalities. This is particularly useful when the abnormality that is sought has prognostic value, e.g. -7 in MDS/AML; +4/+10/+17 in ALL.

2) At follow-up of a patient with a known numerical abnormality, interphase FISH can be used to detect residual disease.

3) Gene amplification can be detected by FISH analysis (e.g. HER2-neu in breast carcinoma or NMYC in neuroblastoma).

d) Translocation/Deletion Detection

1) FISH can be used to detect cryptic rearrangements, such as ETV6/RUNX1 fusion in ALL, FIP1L1-PDGFRA fusion or BCR/ABL1 rearrangements in MPN.

2) FISH can be used to clarify ambiguous or complex rearrangements, or to assist in clarifying rearrangements when morphology is poor, e.g. confirm the involvement of MLL in an 11q23 rearrangement.

3) Interphase FISH can be useful at follow-up to detect specific structural abnormalities such as t(9;22) (e.g. Dewald *et al*., 2000). Analyze a minimum of 200 nuclei.

e) Marker Identification

In individual cases, identification of a marker chromosome may be clinically significant. Paint probes or multicolor FISH techniques can be used in an attempt to identify markers.

C.5 Recommendation for Reporting of Cancer Specimens

The cytogenetics report should describe the chromosome/FISH result, and comment on any change from previous cytogenetic studies. Recommendations for confirmatory FISH tests should be made where appropriate. In addition, interpretive comments about possible diagnoses, prognosis, or other clinical correlations should be made whenever possible.

C.6 Recommendation for Turnaround Time to Completion of Reports

Local policies need to be established and suggested guidelines are listed below. At least 90% of all neoplastic analyses should have final written reports within the recommended turn-around-time (TAT) listed below.

1) STAT (cytogenetics and FISH): depending on the clinical indication, i.e. acute promyelocytic leukemia (APL) for treatment purposes, a preliminary result should be reported within 3 to 5 days with the final written report within 2 weeks.

2) Routine (cytogenetics and/or FISH): at diagnosis – for ALL, AML, and CML final results should be reported within 2 weeks. Results for other neoplastic disorders may be reported within 3 weeks.

3) Routine (cytogenetics and/or FISH): at follow-up - 3 weeks

C.7 Recommendation for retention of case original specimen, slides and cell pellets on cancer specimens

1) Original patient specimen or starting culture material should be retained until the final report has been signed.

2) Disposal of slides should conform to established guidelines for pathology specimens [i.e. DAP (Diagnostic Accreditation Program) or Provincial Accreditation Program].

3) Slides from normal cases may be disposed of after a few months, as per the provincial regulations or other regulating bodies. Slides from abnormal cases should be retained for at least 1 year or as per the laboratory director's discretion.

Cell pellets may represent a valuable resource for additional or supplementary FISH or molecular studies, and should be retained under appropriate conditions to insure the integrity of metaphases, RNA and DNA. It is recognized that there will be financial and space constraints at individual centers.

C.8 References

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