Clinical effect of molecular methods in sarcoma diagnosis (GENSARC): a prospective, multicentre, observational study

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Summary

Background Advances in molecular genetics of sarcoma have enabled the identification of type-specific aberrations. We aimed to assess the clinical effect of systematic implementation of molecular assays to improve sarcoma misdiagnosis.

Methods In this multicentre, observational study, we recruited patients from 32 centres of the French Sarcoma Group/Reference Network in Pathology of Sarcomas. Eligibility criteria included: biopsy or surgical resection; suspicion of: dermatofibrosarcoma protuberans (cohort 1), dedifferentiated liposarcoma (cohort 2), Ewing's sarcoma family of tumours (cohort 3), synovial sarcoma (cohort 4), alveolar rhabdomyosarcoma (cohort 5), and myxoid or round cell liposarcoma (cohort 6); review by one sarcoma-expert pathologist; availability of frozen material (except for cohort 1 of patients with dermatofibrosarcoma protuberans because anti-CD34 immunohistochemistry is performed on paraffin-embedded tissue); and patient information. For each case, the pathologist made one primary diagnosis followed by up to two differential diagnoses, based on histological characteristics only. Each diagnosis was classified as certain, probable, or possible. For each case to determine the molecular classification, we did fluorescence in-situ hybridisation on paraffin-embedded samples. We also did comparative genomic hybridisation and quantitative PCR (cohort 2) or reverse transcriptase PCR (cohorts 3–6) on frozen and paraffin-embedded samples. We made a final diagnosis based on the molecular results. The clinical effect of diagnosis correction was assessed by a board of experts.

Finding Between June 22, 2009, and Oct 30, 2012, 395 patients were enrolled in the study, of which 384 were eligible for inclusion. The diagnosis was eventually modified by molecular genetics for 53 patients: eight (16%) of 50 patients with dermatofibrosarcoma (cohort 1), seven (23%) of 30 patients with dedifferentiated liposarcoma (cohort 2), 13 (12%) of 112 with Ewing's sarcoma family of tumours (cohort 3), 16 (16%) of 97 patients with synovial sarcoma (cohort 4), seven (15%) of 46 patients with alveolar rhabdomyosarcoma (cohort 5), and two (4%) of 49 patients with myxoid or round cell liposarcoma (cohort 6), with an effect on primary management or prognosis assessment in 45 cases.

Interpretation Molecular genetic testing should be mandatory for diagnostic accuracy of sarcoma and appropriate clinical management, even when histological diagnosis is made by pathologist experts in this field.

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Introduction

Sarcomas are a heterogeneous ensemble of rare tumour subtypes with specific histological features.[1] Both rarity and heterogeneity make their diagnosis especially difficult even for pathologists who are experts. The consequence is a high rate of misdiagnosis.[2–4]

An increasing number of specific genetic aberrations including chromosomal translocations, gene amplification, and mutation have been identified in various types of sarcoma. These findings allowed sarcomas to be divided into two main groups: those with defined diagnostic molecular events and those with variable complex genetic changes (appendix p 3). The characterisation of these aberrations during the past 20 years has opened new avenues for molecular diagnosis. At some institutions, some molecular tests are already part of the clinical testing repertoire for diagnosis, especially assays to detect Ewing's sarcoma family of tumours rearrangement in Ewing's sarcomas or *KIT*/*PDGRA* mutational status in gastrointestinal stromal tumours.

Several studies have shown the utility of genetics for the diagnosis of sarcoma subtypes.[5–15] However, most of them were restrospective ones resulting in a biased selection of difficult or second-opinion cases. Moreover, none of them have addressed the clinical effect of genetic tests in terms of therapeutic management and prognostic assessment. Current guidelines suggest pathological diagnosis should be complemented by molecular tests, especially when the specific histological diagnosis is doubtful, the clinical pathological presentation is unusual, or if these tests might have prognostic or predictive relevance.[16] However, such recommendations are based on expert experience and not on published

Research in context

Evidence before this study

Sarcomas represent a heterogeneous group of malignant tumours. Cytogenetic and molecular genetic assays are routinely used for diagnostic and prognostic purposes in molecular pathology laboratories. Many sarcoma subtypes are characterised by recurrent genetic aberrations that can be used as highly specific diagnostic biomarkers. We searched PubMed without restriction in publication dates and language with the terms "sarcoma", "histological review", "diagnoses", "discrepancy", "molecular genetics", "dermatofibrosarcoma", "dedifferentiated liposarcoma", "myxoid/round cell liposarcoma", "Ewing's sarcoma", "alveolar rhabdomyosarcoma", and "synovial sarcoma", to identify retrospective reports relevant to understanding the effect of histological review on accurate diagnosis. At the start of our study in 2008, the existing evidence on the role of molecular tests for accurate diagnosis of sarcoma by experts in the field and their potential impact on patient's prognosis and management were inconclusive. The situation has not changed to date.

Added value of this study

Here, we report that the systematic use of molecular tests allows diagnosis refinement in up to 53 (13%) of 384 cases, even by a pathologist who is an expert in the specialty. In all the cases, diagnosis refinement had an effect on either treatment strategy, prognosis assessment, or both.

Implications of all the available evidence

For all sarcoma with recurrent genetic aberrations, molecular diagnosis test must be done even when the diagnosis is regarded as certain by the sarcoma-expert pathologist.

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See Online for appendix

evidence. Here we report, to our knowledge, the first prospective study assessing the diagnostic, therapeutic, and prognostic effect of molecular analysis in sarcomas.

Methods

Study design and participants

GENSARC is a prospective, multicentre, observational study including 32 centres of the French Sarcoma Group/Reference Network in Pathology of Sarcomas (appendix pp 1–2).[17] This clinical trial aimed to assess the medical and economic effect of the genetic anomalies detection on diagnosis of six types of sarcomas. The initial goal was to include 500 patients. This study included all cases for which an expert pathologist from the French Sarcoma Group considered the diagnosis of dermatofibrosarcoma protuberans (cohort 1), dedifferentiated liposarcoma (cohort 2), Ewing's sarcoma family of tumours (cohort 3), synovial sarcoma (cohort 4), alveolar rhabdomyosarcoma (cohort 5), and myxoid or round cell liposarcoma (cohort 6). Figure 1 shows the inclusion process. Study inclusion criteria were: patient had undergone a biopsy or a surgical resection for a diagnosis or suspicion of sarcoma (cohort 1–6); frozen material was available (except for cohort 1 of patients with dermatofibrosarcoma protuberans because anti-CD34 immunohistochemistry is performed on paraffin-embedded tissue); access to patient clinical record; and having patient information available.

For each case, the pathologist made one primary diagnosis followed by up to two differential diagnoses, based on histological characteristics only. The diagnosis was classified by the pathologist in one of the three following categories when at least one of the six types of sarcoma concerned by the study was called up: certain when it was the only possible diagnosis, probable when it was the most likely hypothesis among other pertinent differential diagnosis, and possible when it represented a potential differential diagnosis. This pre-molecular classification that can be regarded as subjective is pragmatic and reflects the degree of diagnostic certainty that is used in the daily practice and the difficulty of classification of a given histotype.

The diagnosis and category were based on clinical context and histological and immunohistochemical features according to international standards[18] and strictly masked to the genetic test results. For each case, the category of diagnosis was indicated on the inclusion form sent by the pathologist to the coordinator centre. We made a final diagnosis on the basis of clinical context, histology, immunohistochemistry, and molecular results.

An agreement was obtained from ethical committees of each participating institutions. All patients were informed by their clinician and were free to express their opposition to the inclusion in this project in agreement with the French law regarding non interventional study.

Procedures

Upon reception of the histological diagnosis form, the coordinating centre dispatched the molecular analyses to be done by each of the eight molecular centres. For all cohorts, cases were first analysed by a standard method, designated as referent method. This referent method was the most accurate ancillary diagnostic method at the time of the conception of the study in 2008. Additionally, samples were analysed by methods designated as innovative, appendix pp 4–7). For cohort 1 (dermatofibrosarcoma protuberans), the referent method was anti-CD34 immunohistochemistry, for cohort 2 it was comparative genomic hybridisation on arrays (array-CGH) on frozen sample, whereas for cohorts 3–6, the referent method was reverse transcriptase PCR (RT-PCR) on frozen samples. The innovative methods were fluorescence in-situ hybridisation (FISH) detecting translocations (cohorts 1 and 3–6), or *MDM2* amplification (cohort 2), quantitative PCR (cohort 2) and RT-PCR (cohorts 3–6) done on formalin-fixed paraffin-embedded samples. Technical protocols are available on request.