I marcatori biologici (circolanti)

Massimo Gion

Centro Regionale Biomarcatori
AULS12 Veneziana - Regione Veneto

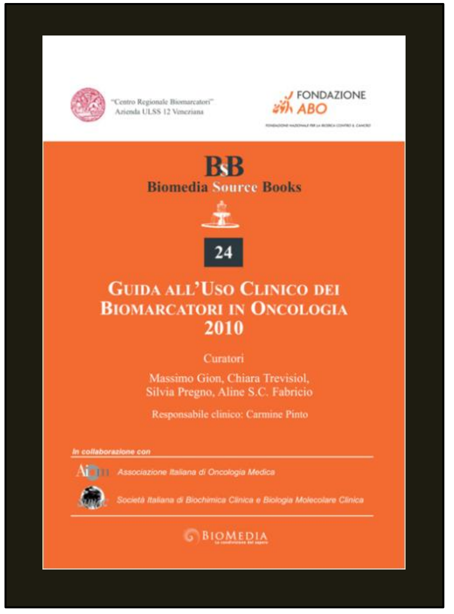
Agenda

1. Lo stato dell’arte: cosa raccomandano le linee guida
2. Lo stato delle cose: cosa facciamo nella pratica clinica
3. Le prospettive: “cosa bolle in pentola”

Raccomandazioni per l’uso clinico: revisione delle evidenze disponibili

Dati dell’aggiornamento (in corso) della

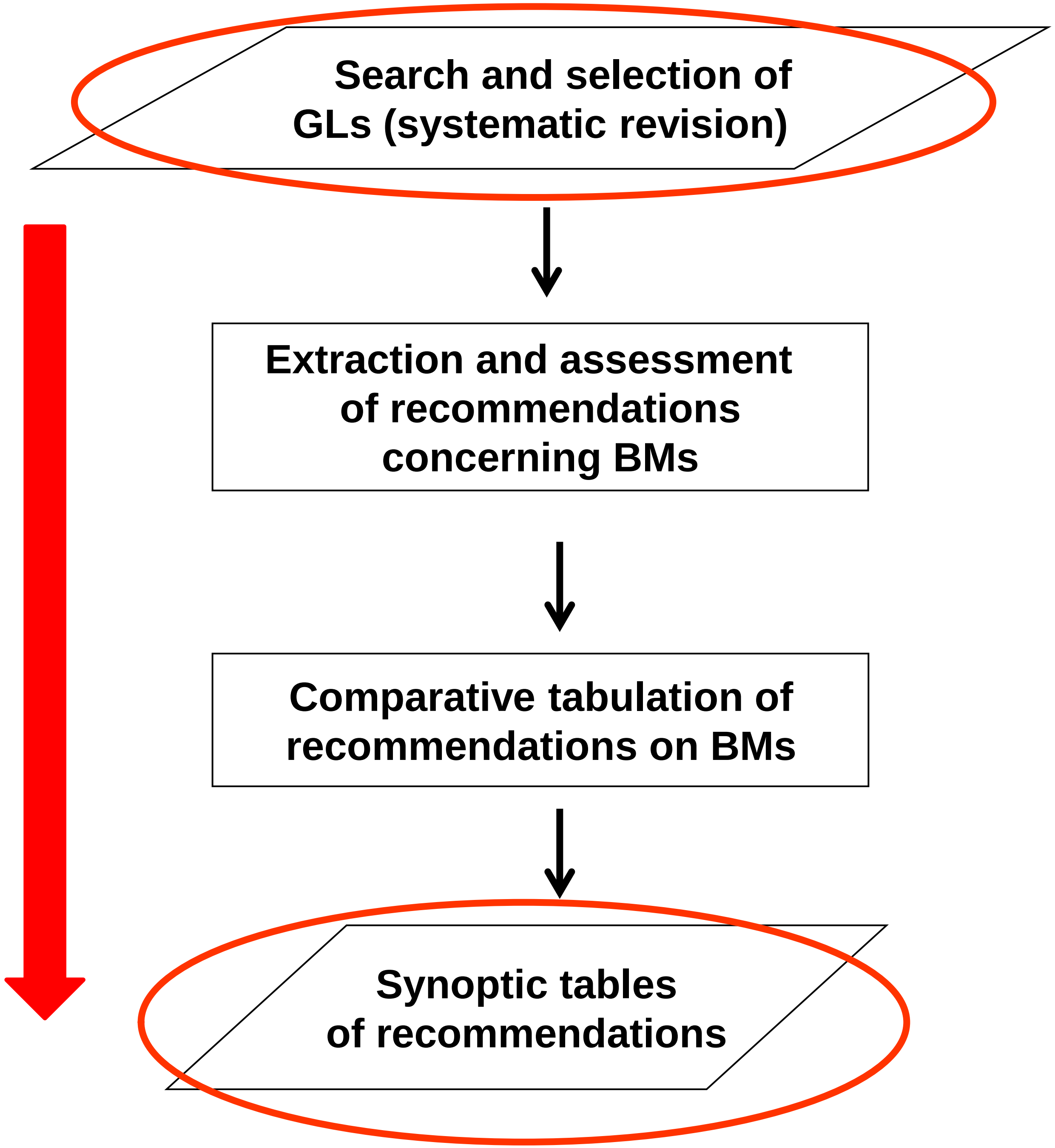
“Guida all’uso Clinico dei Biomarcatori in Oncologia”

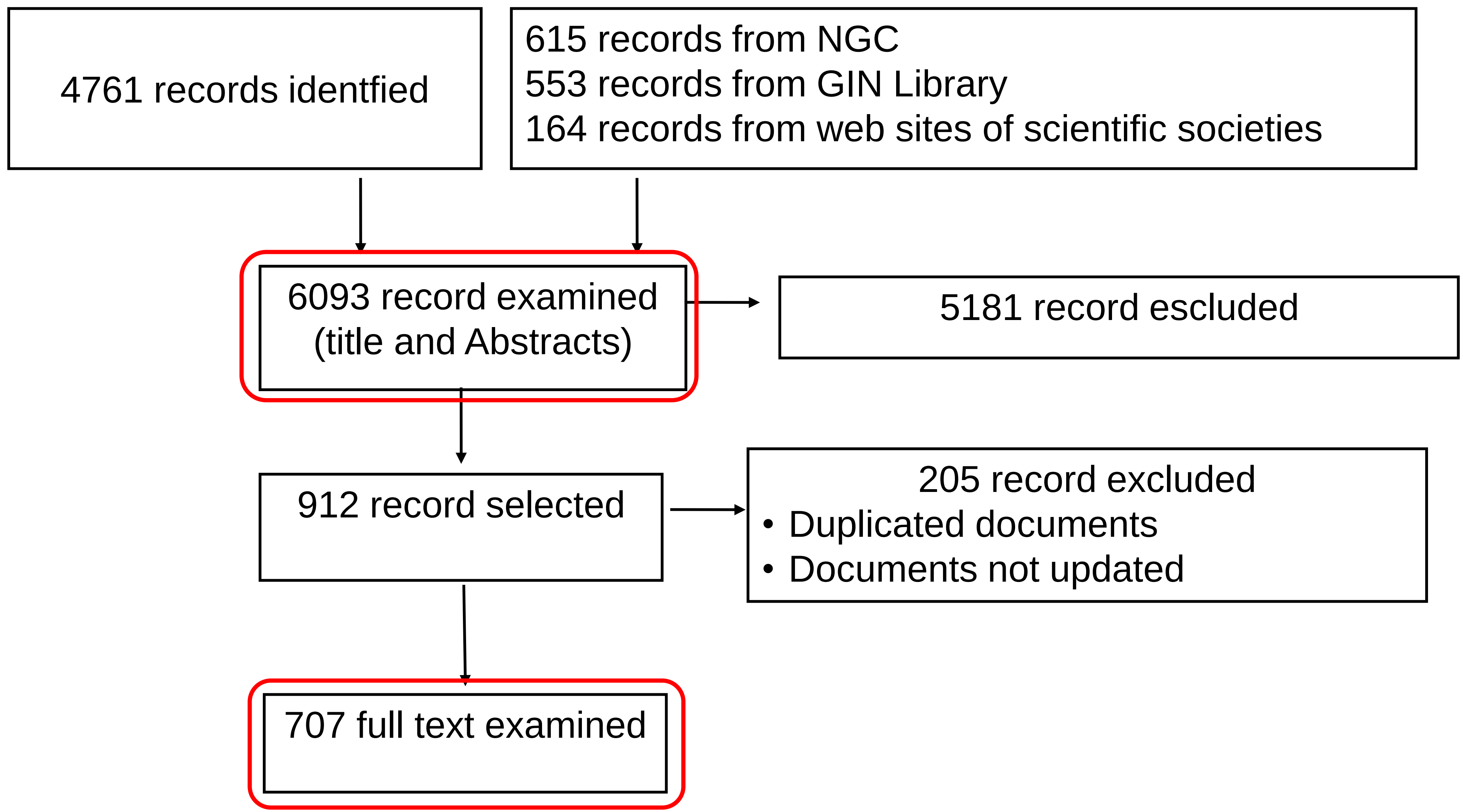


Recomendation for the clinical use: review of available evidence

- Systematic search and appraisal of Clinical Practice Guidelines reporting on ‘tumor markers’, and synoptic presentation of recommendations
- Date of publication: 2008-2014
- Sources - Literature database: MEDLINE (PubMed); Guidelines repository (National Guideline Clearinghouse); Guidelines producers (NICE, SIGN, CCO, NHMRC, SNLG); Scientific societies (AIOM, ESMO, ASCO, NCCN, START, NACB, EGTM, CNR)

- **Promoters**:
  - Centro Regionale Biomarcatori, Azienda ULSS12, Venezia
  - AGENAS (National Agency for Regional Health Services
  - Società Italiana di Biochimica Clinica (SIBioC)
  - Associazione Italiana di Oncologia Medica (AIOM)
- **Responsible of evidence based methodology:**
  - H. Schünemann and A. Rutjes, GRADE Working Group
- **Participants:**
  - 71 researchers and experts from 9 scientific societies


Search and selection of GLs (systematic revision)
Extraction and assessment of recommendations concerning BMs
Comparative tabulation of recommendations on BMs
Synoptic tables of recommendations


4761 records identfied
615 records from NGC
553 records from GIN Library
164 records from web sites of scientific societies
6093 record examined
(title and Abstracts)
5181 record escluded
912 record selected
205 record excluded
• Duplicated documents
• Documents not updated
707 full text examined

Cancer site	References on BC			
Breast	65			

		1° level Assessment of references		
Cancer site	References on BC	Out of topic		
Breast	65	9		

		1° level Assessment of references		
Cancer site	References on BC	Out of topic	Duplicated or not updated	
Breast	65	9	19	

		1° level Assessment of references		
Cancer site	References on BC	Out of topic	Duplicated or not updated	**Identified GLs**
Breast	65	9	19	**37**

Identified guidelines were further selected according to the “Revised (2013) Criteria for Inclusion of Clinical Practice Guidelines in National Guideline Cleringhause”

Documentation of each of the following features in the clinical practice guideline or its supporting documents:

- An explicit statement that the clinical practice guideline was based on a systematic review.
- A description of the search strategy that includes a listing of database(s) searched, a summary of search terms used, the specific time period covered by the literature search.

Cancer site	Identified GLs			
Breast	37			

		Assessment of GLs on the basis of methodological criteria		
Cancer site	Identified GLs	Selected GLs		
Breast	37	**12**		

		Assessment of GLs on the basis of methodological criteria		
Cancer site	Identified GLs	Selected GLs		Excluded GLs
Breast	37	**12**		15

		Assessment of GLs on the basis of methodological criteria		
Cancer site	Identified GLs	Selected GLs	GLs considered even if they do not fulfill criteria (1)	Excluded GLs
Breast	37	**12**	**10**	15

(1) Widely used GLs have been considered also when methodology was not fully compliant with NGC criteria

Cosa dicono le Linee Guida
(Quali, quanti, quando)

1. Screening
2. Diagnosi differenziale
3. Bilancio di base
4. Follow-up
5. Monitoraggio terapia malattia avanzata

Screening

Linee guida che valutano lo scenario: 3/22

Sintesi delle Raccomandazioni	LG	Note
Scenario valutato, marcatori non considerati o nessuna raccomandazione sui marcatori	3/3	---

Diagnosi differenzale

Linee guida che valutano lo scenario: 6/22

Sintesi delle Raccomandazioni	**LG**	**Note**
Scenario valutato, marcatori non considerati o nessuna raccomandazione sui marcatori	6/6	---

Bilancio di base

Linee guida che valutano lo scenario: 9/22

Sintesi delle Raccomandazioni	**LG**	**Note**
Marcatori non raccomandati	2/9	CA15-3, CEA have no value as predictive factor
Scenario valutato, marcatori non considerati o nessuna raccomandazione sui marcatori	7/9	---

Follow-up

Linee guida che valutano lo scenario: 13/22

Sintesi delle Raccomandazioni	**LG**	**Note**
Marcatori non raccomandati nelle pazienti asintomatiche	4/13	Marcatori considerati: CA15.3, CEA
Scenario valutato, marcatori non considerati o nessuna raccomandazione sui marcatori	9/13	---

Monitoraggio terapia malattia avanzata

Linee guida che valutano lo scenario: 9/22

Sintesi delle Raccomandazioni	**LG**	**Note**
Marcatori raccomandati	3/9	Non utilizzabili da soli per decisioni cliniche
Marcatori raccomandati nelle pazienti con malattia non misurabile	1/9	---
Scenario valutato, marcatori non considerati o nessuna raccomandazione sui marcatori	5/9	---

Carcinoma della mammella
Sintesi delle evidenze

- Scenari in cui i marcatori (BM) sono considerati → 3/5
- Scenari in cui i BM sono raccomandati → 1/5
- Scenari in cui i BM <u>non</u> sono raccomandati → 2/5
- Scenari con raccomandazioni concordanti sui BM → 3/3
- BM considerati → 2 (CA15.3 e CEA)

Linee Guida

- La qualità metodologica delle linee guida (LG) è generalmente scadente
- Le LG forniscono poche raccomandazioni sui MT
- Le raccomandazioni non rispondono a tutti i quesiti clinici che si incontrano nella pratica clinica

Linee Guida

- La qualità del contenuto informativo delle LG deriva dalla qualità degli studi primari disponibili

La bassa qualità del contenuto informativo delle LG può indurre inappropriatezza?

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Ricognizione della prescrizione da parte dei MMG

- Metodo di monitoraggio indiretto dell’appropriatezza su larga scala: confronto fra dati di prescrizione (esami fatti) e dati epidemiologici (esami attesi)
- Dati di prescrizione per utenti ambulatoriali di tutte le regioni; fonte Min Sal
- Dati epidemiologici: fonte AIRTUM (Rete dei Registri Tumori Italiani)

DE GRUYTER DOI 10.1515/cclm-2013-0708 — Clin Chem Lab Med 2014; x(x): xxx–xxx

Massimo Gion*, Roberta Franceschini, Claudia Rosin, Chiara Trevisiol, Lucia Peloso, Marco Zappa, Aline S.C. Fabricio and on behalf of the Working Group on Tumour Markers of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC)

An epidemiology-based model to estimate the rate of inappropriateness of tumor marker requests

Totale popolazione e marcatori eseguiti

Fonte dati: Nsis - Flusso di specialistica ambulatoriale Art 50 (Legge 326/2003)

	2012
Popolazione Italiana	59.685.227
Tot. marcatori prescritti	

Totale popolazione e marcatori eseguiti

Fonte dati: Nsis - Flusso di specialistica ambulatoriale Art 50
(Legge 326/2003)

	2012
Popolazione Italiana	59.685.227
Tot. marcatori prescritti	13.207.289

Totale popolazione e marcatori eseguiti

Fonte dati: Nsis - Flusso di specialistica ambulatoriale Art 50 (Legge 326/2003)

	2012
Popolazione Italiana	59.685.227
Tot. marcatori prescritti	13.207.289
Tot marcatori prescritti x 1000 abitanti	221,3

	2012
Tot. marcatori prescritti	13.207.289
CA15.3	1.078.864

Tavolo Interregionale Biomarcatori - Commissione Salute. Dati in corso di elaborazione, 2014-15

Prevalenza, Italia 1 gennaio 2006

522 235 persone hanno avuto una diagnosi di **tumore della mammella** femminile, nel corso della vita.

Di queste	**82 117**	l'hanno avuta da meno di 2 anni	(16%)
	108 338	da 2-5 anni	(21%)
	131 591	da 5-10 anni	(25%)
	86 647	da 10-15 anni	(17%)
	53 812	da 15-20 anni	(10%)
	59 732	l'hanno avuta da più di 20 anni	(11%)

16%	21%	25%	17%	10%	11%
82 117	**108 338**	**131 591**	**86 647**	**53 812**	**59 732**
≤ 2 y	2-5 years	5-10 years	10-15 years	15-20 years	> 20 years

	2011	2012
CA 15.3	1.078.864	1.112.719

Carcinoma della mammella: prevalenza

Totale (stima per l'anno 2006) **522.235** donne

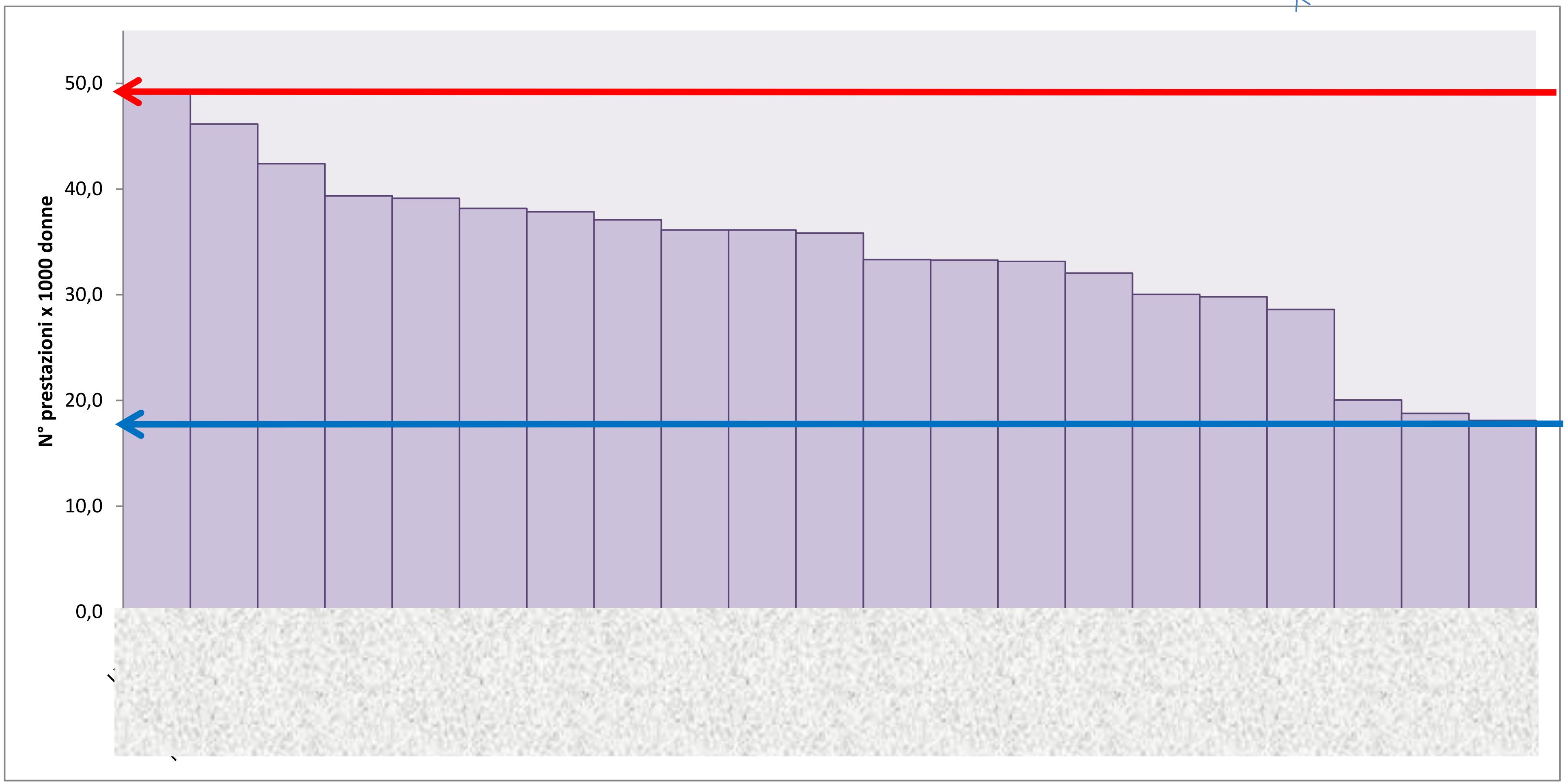
Prevalenza donne che hanno ricevuto una diagnosi:

da meno di 2 anni	**83.557**	(16%)	**323.783**
tra i 2 e 5 anni	**109.669**	(21%)	
tra i 5 e 10 anni	**130.559**	(25%)	
oltre i 10 anni	**198.450**	(38%)	

Le variazioni regionali

Analisi per regione del CA15.3

CA15.3 - Variazioni di richieste fra le diverse Regioni. Il tasso di richieste varia da un minimo di 19 a un massimo di 50/1000 donne.



La "costanza" prescrittiva

Neoplasia bersaglio			
Ca Mammella			
Ca Ovaio + endometrio			
Ca pancreas + vie biliari			

La “costanza” prescrittiva

Neoplasia bersaglio	**Prevalenza (Italia, 2010)**		
Ca Mammella	522.235		
Ca Ovaio + endometrio	129.515		
Ca pancreas + vie biliari	18.755		

La “costanza” prescrittiva

Neoplasia bersaglio	Prevalenza (Italia, 2010)	Marcatore specifico	
Ca Mammella	522.235	CA15.3	
Ca Ovaio + endometrio	129.515	CA125	
Ca pancreas + vie biliari	18.755	CA19.9	

La “costanza” prescrittiva

Neoplasia bersaglio	Prevalenza (Italia, 2010)	Marcatore specifico	Prescrizioni (Italia, 2012)
Ca Mammella	522.235	CA15.3	1.078.864
Ca Ovaio + endometrio	129.515	CA125	
Ca pancreas + vie biliari	18.755	CA19.9	

La “costanza” prescrittiva

Neoplasia bersaglio	Prevalenza (Italia, 2010)	Marcatore specifico	Prescrizioni (Italia, 2012)
Ca Mammella	522.235	CA15.3	1.078.864
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La “costanza” prescrittiva

Neoplasia bersaglio	**Prevalenza (Italia, 2010)**	**Marcatore specifico**	**Prescrizioni (Italia, 2012)**
Ca Mammella	522.235	CA15.3	1.078.864
Ca Ovaio + endometrio	129.515	CA125	977.189
Ca pancreas + vie biliari	18.755	CA19.9	1.386.169

- Il rapporto fra numero di richieste e prevalenza dei diversi tumori nonché le differenze fra le Regioni non sono in alcun modo riconducibili alla epidemiologia dei tumori.
- La modalità di richiesta sembra riflettere prevalentemente abitudini prescrittive locali o regionali e come tale presenta ampi margini di miglioramento.

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La biopsia liquida

- Recently discovered technologies enable the extraction of molecular information on the primary tumor by analyzing tumor-derived genetic material in the bloodstream
- Liquid biopsy approaches have a high potential to give detailed information on tumor genome evolution over time, that can be used for serial monitoring of patients

NEWS FEATURE

WRITTEN IN BLOOD

DNA circulating in the bloodstream could guide cancer treatment —

BY ED YONG

Clinical Chemistry

January 2015

Volu

AACC

La biopsia liquida

Tumor-derived genetic material in blood

- MicroRNA (miRNA)
- Cell-free circulating tumor DNA (ctDNA)
- Circulating Tumor Cells (CTC)

Clinical Chemistry 61:1
56–63 (2015)

Mini-Reviews

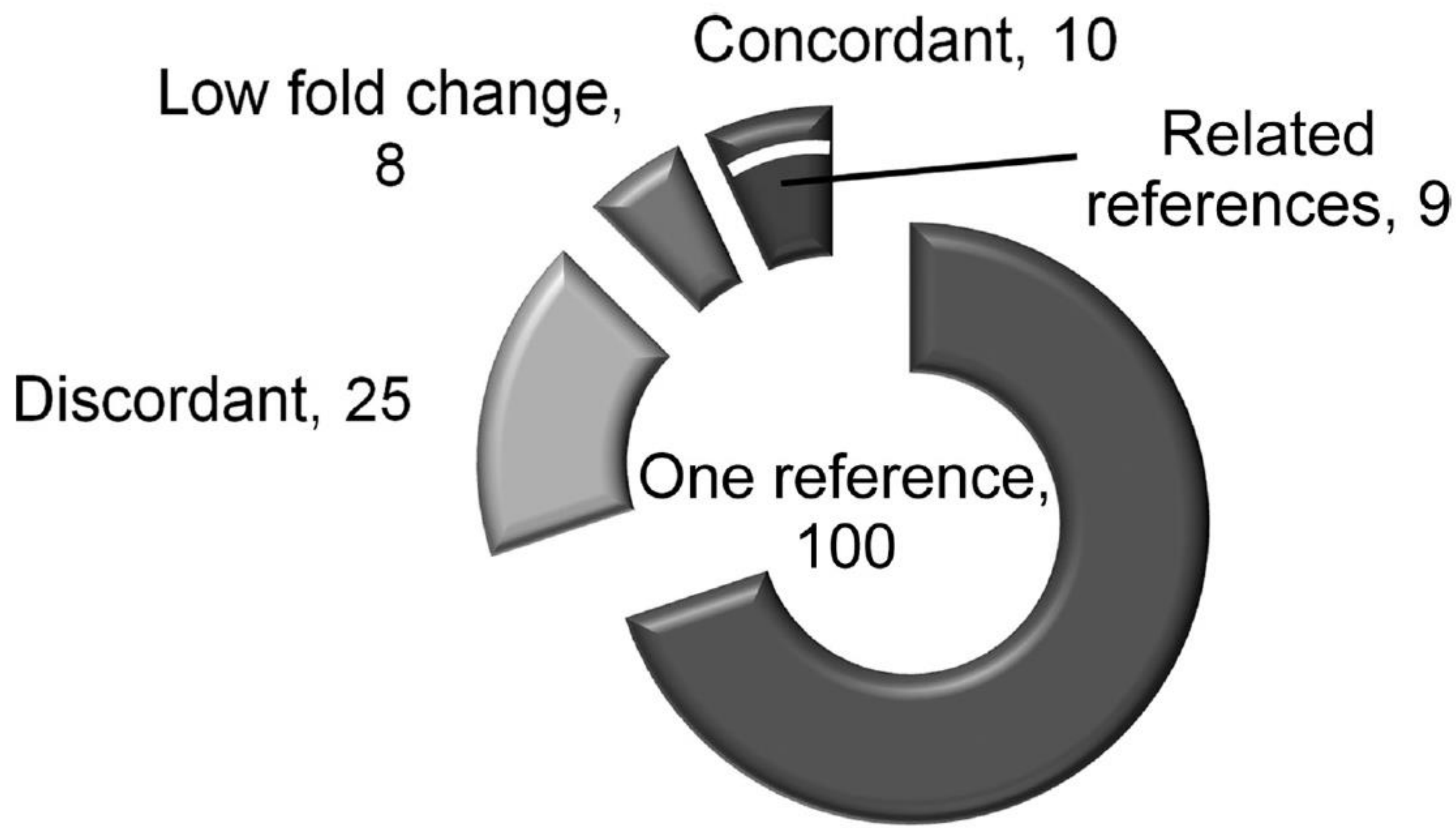
Circulating MicroRNA Biomarker Studies: Pitfalls and Potential Solutions

Kenneth W. Witwer[1]*

- Commonly reported miRNA biomarkers are largely nonspecific, associated with a wide range of conditions and outcomes.
- Limited overlap has been observed between the findings of even very similar studies of the same disease.

(Witwer KW, Clin Chem 2015)

Of 143 circulating miRNAs reported to be differentially regulated ….



(Witwer KW, Clin Chem 2015)

- Most extracellular miRNAs in circulation have a blood or endothelial cell origin.
- The fraction of extracellular RNA deriving from tissue is unknown and variable, but small.
- The fraction of circulating miRNA from a tumor would depend on the tumor's size.
- To date, circulating miRNA research has not resulted in validated disease markers.

(Witwer KW, Clin Chem 2015)

La biopsia liquida

Tumor-derived genetic material in blood

- MicroRNA (miRNA)
- Cell-free circulating tumor DNA (ctDNA)
- Circulating Tumor Cells (CTC)

Clinical Chemistry 61:1
112–123 (2015)

Reviews

Circulating Tumor DNA as a Liquid Biopsy for Cancer

Ellen Heitzer,[1*] Peter Ulz,[1] and Jochen B. Geigl[1]

Approaches for the analysis of free circulating DNA

- Simple quantitative assessment
- Targeted approach that includes the analysis of known genetic changes from the primary tumor (i.e. mutations in KRAS or EGFR).
- Untargeted approach without knowledge of any specific changes present in the primary tumor (i.e. Genomewide analysis of ctDNA)

(Heitzer E et al, Clinical Chemistry 61:1; 112–123, 2015)

- Because cell-free circulating tumor DNA (ctDNA) is a potential surrogate for the entire tumor genome, the use of ctDNA as a liquid biopsy may help to obtain the genetic follow-up data
- For actual implementation of the ctDNA in clinical practice, it is necessary to develop standardized preanalytical and analytical methodologies, including blood collection, processing, and storage and DNA extraction, quantification, and validation in large prospective clinical studies

(Heitzer E et al, Clinical Chemistry 61:1; 112–123, 2015)

La biopsia liquida

Tumor-derived genetic material in blood

- MicroRNA (miRNA)
- Cell-free circulating tumor DNA (ctDNA)
- Circulating Tumor Cells (CTC)

CCR FOCUS

Circulating Tumor Cells: A Multifunctional Biomarker

Timothy A. Yap[1,2], David Lorente[1,2], Aurelius Omlin[3], David Olmos[4], and Johann S. de Bono[1,2]

Clin Cancer Res; 20(10); 2553–68. ©2014 AACR.

Circulating Tumor Cells (CTC)

Enumeration

The U.S. FDA approved baseline CTC counts - assessed with CellSearch technology - as a prognostic marker in metastatic breast (colorectal, and prostate cancers).

Molecular characterization

- HER2 expression
- EGFR expression
- IGF-IR expression
- γ-H2AX expression and other markers of apoptosis
- ….

Because CTCs are extremely rare cells in the bloodstream, enrichment techniques have been used for separation from peripheral blood cells, including:

- Immunomagnetic assays: positive selection EpCAM antibodies
- Immunomagnetic assays: negative selection CD45 antibodies
- Microfluidic chips
- Size
- Size and deformability
- Density
- Immunomagnetic and physical properties
- Dielectrophoretic
- Other

(Yap TA, Clin Cancer Res 2014)

Identification of CTCs is then performed, through either:

- immunofluorescence,
- reverse transcription PCR (RT-PCR),
- other techniques involving sophisticated software and microscopy

(Yap TA, Clin Cancer Res 2014)

frontiers in
ONCOLOGY

MINI REVIEW ARTICLE
published: 26 February 2015
doi: 10.3389/fonc.2015.00042

Clinical implications of circulating tumor cells of breast cancer patients: role of epithelial–mesenchymal plasticity

Linda M. McInnes[1†], Natalie Jacobson[1†], Andrew Redfern[2], Anthony Dowling[3], Erik W. Thompson[4,5,6‡] and Christobel M. Saunders[1*‡]

[1] School of Surgery, The University of Western Australia, Perth, WA, Australia
[2] Medical Oncology, Royal Perth Hospital, Perth, WA, Australia
[3] Department of Medical Oncology, St Vincent's Hospital Melbourne, Melbourne, VIC, Australia
[4] Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia
[5] St. Vincent's Institute, Melbourne, VIC, Australia
[6] Department of Surgery, St Vincent's Hospital, University of Melbourne, Melbourne, VIC, Australia

- Although CTC count changes are predictive of outcome in MBC, this is largely a disease where serial agents are delivered with palliative intent. Hence, early tailoring of therapies may not greatly impact on outcome.
- To date, clinical trials have shown that absolute CTC count alterations or CTC persistence do not predict strongly for neoadjuvant response, or improved adjuvant or metastatic outcomes, and hence currently do not provide clinically useful information to drive changes in therapies.
- With the maturation of the current clinical trials and further developments in the molecular characterization of CTCs, this information will hopefully become available

(McInnes LM et al., Front. Oncol, 5:42. doi: 10.3389, February 2015)

A selection of current ongoing clinical trials examining the clinical utility of CTCs in breast cancer treatment.

Trial name (ClinicalTrial.gov registry number)	Rationale	Patient group	Methodology	Estimated accrual completion date
CTC-EMT (NCT02025413)	Evaluating a novel mesenchymal-marker-based ferrofluid (N-cadherin or O-cadherin based) CTC capture method.	Metastatic prostate or MBC patients	Non-randomized study to evaluate novel CTC capture method.	December 2014
STIC CTC METABREAST (NCT01710605)	Evaluating the medico-economic value CTCs provide in deciding on first-line therapy.	HR+, HER2− MBC patients	Randomized study where patients with ≥5 CTC/7.5 ml blood receive chemotherapy and those with <5 CTC/7.5 ml receive endocrine therapy.	February 2015
COMETI P2 (NCT01701050)	Evaluating the algorithm CTC-Endocrine Therapy Index (CTC-ETI) for the identification of patients that will progress.	ER+, HER2− MBC patients	ER, B-cell lymphoma-2 (BCL2), HER2, and Ki-67 markers assessed on isolated CTCs and CTC-ETI determined.	December 2015
Treat-CTC (NCT01548677)	EBC, HER2− primary tumor patients with no overt metastasis having completed (neo) adjuvant chemotherapy and surgery.	HER2−, CTC+ EBC patients	Patients randomized in 1:1 ratio to either the trastuzumab arm or the observation arm.	April 2017
CTC-CEC-AND (NCT02220556)	Evaluation of different analysis methods for CTCs, CECs, and circulating tumor DNA in patient followed for a tumoral pathology.	Patients with solid tumors	Fifteen cohorts. Each cohort will test one analysis method and/or tumoral type. Up to 50 patients in each cohort.	December 2015
CirCe01 (NCT01349842)	Evaluation of the use of CTCs to guide chemotherapy from the third-line of chemotherapy for MBC.	Advanced MBC patients	Patients with ≥5 CTCs/7.5 ml before third-line of chemotherapy randomized between CTC-driven and standard treatment.	January 2018
DETECT III (NCT01619111)	A multicenter, phase III study to compare standard therapy ± Lapatinib in HER2− MBC patients with HER2+ CTCs.	HER2− MBC patients with HER2+ CTCs	Patients randomized between standard therapy ± Lapatinib. Patients with bone metastases treated with denosumab.	March 2018

(McInnes et al., Front. Oncol, 5:42. doi: 10.3389, February 2015)

- The real challenge of CTC technologies is to monitor minimal residual disease in patients without signs of overt metastasis, as the CTC counts are very low in these patients.
- more sensitive methods and/or the analysis of larger amounts of blood might be required to increase the robustness of CTC measurements in these patients.
- The implementation of leukapheresis or other innovative approaches for CTC enables the screening for CTCs in blood volumes of 1.5-25 liters.

(Joosse SA, Gorges TM Pantel K. EMBO Molecular Medicine 7/1, 2015)

Clinical Chemistry 60:10
1249–1251 (2014)

Editorial

Molecular Characterization of Circulating Tumor Cells: Holy Grail for Personalized Cancer Treatment?

Evi S. Lianidou[1*]

- Preanalytical issues and analysis steps should be carefully standardized and subjected to thorough internal and external quality control systems.
- Moreover, validation of clinical utility of CTC analysis assays through defined endpoints, such as overall survival, in multicenter clinical trials is <u>absolutely</u> necessary ….
- … <u>before</u> their implementation in clinical practice.

(Lianidou ES, Clinical Chemistry, 2014)

2015 - I biomarcatori sembrano essere in un cul-de-sac

- I marcatori circolanti tradizionali hanno bassa evidenza a supporto, sono molto utilizzati, ma probabilmente scarsamente usati per decisioni cliniche
- I biomarcatori tissutali sono utili in prima linea, ma perdono efficacia nel monitoraggio longitudinale (eterogeneità iniziale del tumore, variazioni nel tempo di genotipo e fenotipo, …)
- Alcuni nuovi marcatori circolanti sono promettenti, ma non utili negli scenari critici

Conclusioni e prospettive (1)
Livello ricerca

- Condurre solo studi di larghe dimensioni e disegno adeguato
- Definire per i marcatori obiettivi significativi in termini di utilità (servirà nella pratica clinica?) e non solo di validità (funziona?)
- Valutare con competenze multidisciplinari gli aspetti preanalitici (il campione) e analitici (il metodo) (non delegare al committente la gestione delle analisi!)

Conclusioni e prospettive (2)
Livello pratica cinica

Agire con saggezza!

- Modificherò le mie decisioni cliniche sulla base del risultato del marcatore che sto per chiedere?
- Ho prove che la mi scelta produca poi più benefici che rischi o danno al paziente?

Grazie per l’attenzione

Critical areas in the biomarker pipeline

1. Biospecimens
2. Analytic Performance
3. Standardization and Harmonization
4. Bioinformatics
5. Collaboration and Data Sharing
6. Regulatory Issues
7. Stakeholder Education and Communication
8. Science Policy

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

5. Collaboration and Data Sharing

- Form a model of pre-competitive consortium to facilitate sharing scientific information and research operations
- Develop incentives to encourage collaborations among drug sponsors, clinical research sponsors, and regulatory authorities
- Encourage contribution of biospecimen data and experimental data to public databases

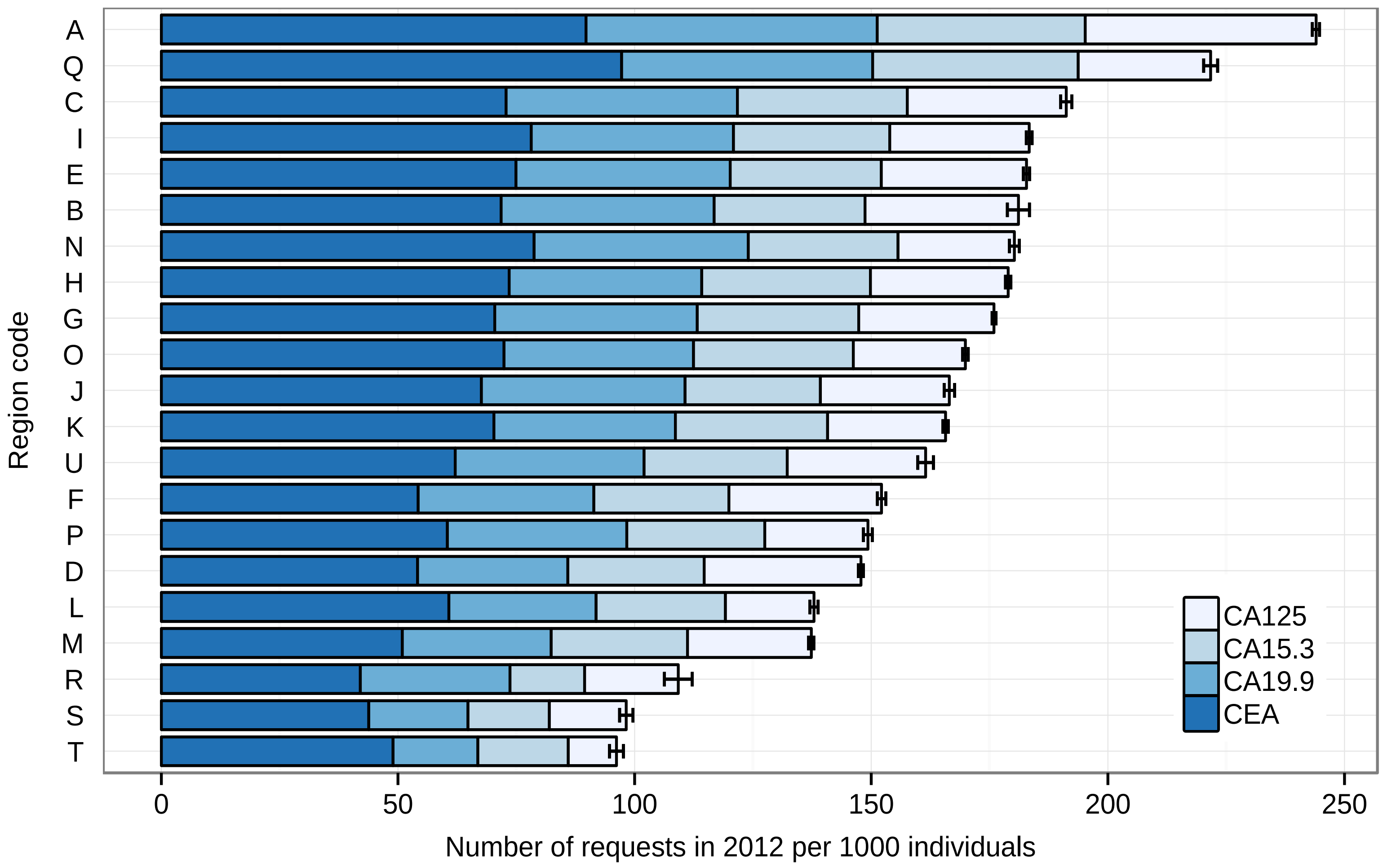
(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

7. Stakeholder Education and Communication

- …
- Educate patients and health care providers about the value and need for biospecimen collection
- Increase awareness and understanding of the importance of analytic validation and quality control
- ….

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

Variazioni di richieste fra le diverse Regioni (indicate da un codice). Il tasso di prescrizione varia da un minimo di 98 richieste/1000 abitanti a un massimo di 239/1000.



Quanti malati di tunore ci sono in Italia?

RAPPORTO AIRTUM 2010 - SINTESI

PERSONE CON TUMORE
2 243 953

Prevalenza, Italia 1 gennaio 2006

2 243 953 persone hanno avuto **una diagnosi di tumore** nel corso della vita.

Di queste	**471 303**	l'hanno avuta da meno di 2 anni	(21%)
	486 969	da 2-5 anni	(22%)
	523 553	da 5-10 anni	(23%)
	312 823	da 10-15 anni	(14%)
	186 704	da 15-20 anni	(8%)
	262 600	l'hanno avuta da più di 20 anni	(12%)

Persone che hanno avuto una diagnosi entro 15 anni

1.794.649

Marcatori prescritti per anno

- per 1000 abitanti: 221,3
- per caso prevalente: 7,4