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Biomarkers in Reproductive Medicine: The promise, and can it be fulfilled?

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Abstract

A biomarker can be used for early diagnosis of a disease, identification of individuals for disease prevention, as a potential drug target, or as a potential marker for a drug response. A biomarker may also limit the use of drug (and therefore costs) to the population of patients where the drug will be safe and efficacious. A biomarker in reproduction could be used to improve assessment of exposure, identify subgroups susceptible to treatment, predict outcome and/or differentiate subgroups with potentially different etiologies of disease. Despite many potential uses there is low participation in reproductive biology to develop molecular biomarkers which may be directly related to the low number of new molecular entities entering clinical trials. As the number of candidate markers in reproductive medicine is increasing, it is important to understand the pathway of development from discovery to clinical utility and recognize that the vast majority of potential markers will not be clinically useful due to a variety of pitfalls. Extensive testing, validation and modification needs to be performed before a biomarker is demonstrated to have clinical utility. New opportunities and partnerships exist and should hasten the development of biomarkers in reproduction. As more biomarkers are moved into practice, a better educated biomarker consumer will enhance the possibility that biomarker(s) will realize their great potential.

Keywords

biomarker; validation; reproduction

What is a biomarker?

The search for relevant biomarkers that diagnose a clinical condition or predict the patient response to a drug has intensified in all therapeutic areas, including the field of reproductive medicine. The recognized importance of biomarkers in health management has led to specific definitions from working groups. The official NIH definition of a biomarker is: *"a*

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characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (1). The characteristics a biomarker measures are varied, and can include morphology, imaging technologies such as X-rays or MRI, and genetic markers or molecular entities, which are the primary focus of this edition. A molecular biomarker should be easily obtainable, have an assay that will provide a rapid result and reflect a disease process where non-invasive or early detection is of clinical benefit. A biomarker can be used for early diagnosis of a disease, identification of individuals for disease prevention, as a potential drug target, or as a potential marker for a drug response (2, 3). Alternatively a biomarker can be used to detect recurrence or progression of a disease or prognosis. A biomarker may also direct the diagnostic strategy to decreased complexity or cost. A molecular biomarker is not of value if it only detects late stage disease that can already be identified by exam, imaging modality, or other clinical tests. This issue of Fertility and Sterility is devoted to biomarkers in reproductive medicine. At the outset of this issue, it seems worthwhile to define the customers who utilize and benefit from biomarkers.

The consumers of biomarker research can be divided into at least two categories. The first category is the extended medical community that publishes and reads the scientific literature. The second category is engineers (design fluid handling or detection systems) or biostatisticians (transform comprehensive biomarker knowledge into essential analytes) that develop biomarkers into a product for health care. Often, but not always, these consumers overlap. These two consumer groups become the new suppliers of biomarker technologies for health care. Only recently have these two groups collaborated to supply innovations to health care customers. Much of what is published in the scientific literature is never developed into a therapeutic or diagnostic product, and sometime the data leading to the development of a product is never published. However, the goals and pitfalls of the development of a biomarker overlap, and both are covered in this dedicated journal edition and in this review.

Utility and pitfall of the use of biomarkers in clinical practice

Concomitant with the increase in discovery of biomarker, there must be education on how markers will be used in clinical medicine. Unfortunately there is no paradigm that applies to the clinical use of a biomarker in general. The use of each biomarker needs to be individualized. The link of a biomarker to the underlying biological process is not a requisite for the marker to have clinical utility. However, connecting the mechanistic dots of a marker to a condition will likely increase clinical uptake. Alternatively a biomarker developed along a putative etiologic line also has drawbacks. A false assumption that there is a universal mechanism of disease etiology, or progression, will invariably lead to poor utility in complex diseases (such as sub-fertility) or in diverse populations. A biomarker may be of great utility for a subgroup, but not for all. For example, the detection of a chlamydia antibody is not a good biomarker for all forms of tubal disease. Egg quality is not solely a function of the paracrine and endocrine function of the granulosa cell; it is possible that a woman can have “decreased ovarian reserve” and still have a normal AMH.

A common reason for a biomarker to fail is that it may be associated with one aspect of a disease, but not the aspect of clinical importance. A biomarker for endometriosis based on inflammation may be of limited value if pain is not associated with generalized inflammation (but instead some other process). Another example is that of putative biomarkers of *in vitro* embryonic development. The pace of cell division, or metabolism of an embryo in vitro, does predate implantation and thus may be informative as a biomarker. However, implantation and the development of an early pregnancy are also strongly associated with maternal factors which are still not completely understood. Thus, the

association between cell division and implantation may be strong, but insufficient to incorporate myriad clinical factors that influence conception after embryo transfer. At the very least the limitations of prediction (the intended use) of any biomarker must be clearly established and understood by potential users.

Even when a biomarker has clear utility and a strong association, there needs to be consideration regarding how it is to be developed and ultimately its “intended use”. A noninvasive diagnostic test replacing the need for an expensive surgical intervention has intuitive benefit. However, there is often disagreement which diagnostic test characteristics should be optimized. In the case of endometriosis, fibroid or even ectopic pregnancy, it is not clear if it is preferable to maximize sensitivity or specificity (4, 5). If sensitivity is maximized more women with disease will be diagnosed, but a larger number of healthy women will be falsely diagnosed and perhaps treated. If specificity is maximized more healthy women will be diagnosed correctly as disease free, but a larger number of women with disease will be falsely diagnosed as healthy and perhaps not receive treatment and it benefits.

Receiver operator curves are often used to assess the utility of a diagnostic test. The area under the curve can be compared statistically. Such a summary measurement can be misleading as the area under the curve assumes a maximization of both sensitivity and specificity. In clinical terms, however, the relative importance of an error of false diagnosis may differ depending on disease of interest, and may not be equal. The balance of sensitivity and specificity will depend on both the disease of interest and the potetila biomarker. Finally, accuracy and predictive value are also very important test characteristics and should be reported in any presentation of data regarding the development of a biomarker.

There is also long standing debate about the appropriate (and inappropriate use) use of biomarkers. One such example is the use of biomarker for ovarian reserve. An elevated FSH has good predictive value in “high risk” population (women in their later thirties or older, or who have poor response, or success, with IVF). However, basic epidemiological principals demonstrate that when the screening test is used in “low risk” women (those curious about their fertility potential, or less than age 35) the predictive value drastically decreases to levels where an unacceptable proportion of women are falsely labeled as unable to achieve pregnancy, and at times are denied care (with their own eggs) (6).

One must also be mindful that a biomarker is not always a surrogate endpoint and may not provide assessment of risk. Androgens are an important biomarker of polycystic ovary syndrome (PCOS) and are part of the diagnostic criteria. However, androgen levels are not a good surrogate for associated health outcomes in women with PCOS. For example, manipulation of serum androgen concentrations has not been demonstrated to reduce cardiovascular disease (7). The goal of clinical medicine is to reduce mobility and mortally, not optimize the level of a biomarker.

Pharmaceutical and Healthcare businesses anticipate greater use of biomarkers

Pharmaceutical development envisions that the drug consumer is the patient, but that the person influencing treatment decisions is the physician prescriber, with recommendations from their formularies. For biomarkers, besides the patient, the primary customer is the managed health care system. The objective of a biomarker is to limit the use of drug (and therefore costs) to the population of patients where the drug will be safe and efficacious. Therefore the most effective healthcare spend is when the drug is prescribed in conjunction

with a highly predictive biomarker for a selected patient population, where it is effective and safe.

In 2010, a report from UnitedHealth estimated that Medicare payments for clinical laboratory services totaled $8.1 billion (8). The estimated costs for genetic and molecular diagnostics among UnitedHealth participants in 2010 totaled $5 billion, representing 8 percent of national spending on clinical laboratory services. UnitedHealth estimates for the insured clinical laboratory services for genetic and molecular testing are expected to reach $25 billion by 2021. This represents 500% growth over the next 12 years. This clearly identifies an emerging medical opportunity for genetic and molecular testing (8).

While this report establishes the need for biomarker testing exists, only 4% of physicians surveyed had ordered genetic tests for their patients while 75% of the physicians believed that genetic testing would benefit the personalized care of their patients (8). However, only 7% of physicians were very knowledgeable about genetic science, and 16% admitted to being not knowledgeable (8). Among the 1,000 to 3,000 new complex biomarker tests that are being developed, only a minority of the tests have clinical data that validates the intended use claim. It can quickly become obvious that if only 7% of physicians are very knowledgeable about biomarker tests, and there are increasing numbers of tests available, there is a need to validate the value of new genetic or molecular diagnostic tests in cost effective clinical research.

Biomarkers in Reproduction

While biomarker research has been conducted for decades it has enjoyed resurgence in popularity and prevalence potentially due to advances in the “omics”. High-through-put platforms can generate large amounts of data. It is both intriguing and relatively easy to propose association between putative or novel marker and disease process. The difficulty is validating such an association, and moving it into the clinical arena. To date the field of reproductive medicine has lagged behind other fields of medicine in its implementation of biomarkers into clinical medicine, but based on the pace of publication that gap may be closing.

Three summary points are relevant for this review: 1) there is low participation in reproductive biology areas for clinical studies to develop molecular biomarkers of disease, or of pharmacogenomic response; 2) the lack of pharmacogenomic markers is directly related to the low number of new molecular entities entering clinical trials (9); and 3) few of clinical investigators design pharmacogenomic studies with molecular biomarkers. So why is there a paucity of use of biomarkers in reproduction when the potential is so high?

The biomarker clinical validation process

The identification and development of a biomarker for an intended use in the clinic has distinct milestones (2, 10, 11, 12). The first milestone is identification of promising markers from preclinical exploration. This often happens early in the preclinical drug discovery process or in clinical research, and delivers either (a) an assessment of putative markers based on our understanding of disease mechanism or (b) unbiased candidate biomarker discovery. The second milestone is the validation of the initial findings with a clinical assay that replaces the biomarker discovery assay, in a similar patient population used for the first milestone. The third milestone is to demonstrate that the clinical biomarker assay performs consistent with its intended use (detect disease, stratify patients, etc.) often with a longitudinal or retrospective cohort (2, 10, 11). The goal is to verify that the marker(s) detect(s) disease, or identifies patient subpopulations preferably early in the course of the disease. The fourth milestone is to validate that the biomarker performs according to its

intended use, usually in a prospective screening to identify the extent or characteristics of a disease when detected by tests. Documents that are prepared for FDA to register the biomarker kit follow either a predicate device (substantially equivalent device exists), a 510(k) registration for devices that contain new components, or a premarket approval (PMA) process for novel devices. These documents for biomarkers are most often filed after the third or fourth milestone. Ultimately, the goal of biomarker development is to improve outcome of clinical care and/or reduce costs.

The approach to biomarker development in reproduction to date, at best, can be considered uncoordinated and disparate. As will be clearly recognized upon reading the articles in this journal, potential biomarkers in reproductive medicine are in various stages of development. The most likely disposition for a promising new biomarker is that it will never be validated. Often a biomarker is presented as a novel finding (milestone 1 achieved) and no further data is ever published; akin to a one hit wonder. It is possible that there was never an attempt to validate a marker, or the utility of the marker fails to reach the second milestone, and these data are often unpublished. Often the use of a predictive biomarker has poorer test characteristics when it is validated in a separate population, especially in a population distinct from its development (13). As example, multiple diagnostic markers for endometriosis have been proposed, but have not been validated in populations, distinct from its development (4). Finally it is possible for a marker to proceed to large scale prospective testing and be found to have limited clinical utility, e.g. for embryo viability assessment (14). Common pitfalls in development of biomarkers are presented in Figure 1. Some factors include issues regarding phenotyping of samples, collection and storage of biomaterials, novel assays, confounding, and over interpretation of chance finding (14, 16). The issue of false finding is of particular concern when analyzing large amounts of data that are generated from genomic, proteomic or metabolomic screening. All of these issues exemplify the importance of external validation using an independent sample.

Need for standardization

The field of biomarker identification is rapidly evolving. The quality of reporting of studies of diagnostic accuracy is less than optimal in general, and the field of reproduction is not an exception. Complete and accurate reporting is necessary to enable readers to assess the potential for bias in the study and to evaluate the conclusions made from the results. A group of scientists and editors has developed the STARD (Standards for Reporting of Diagnostic Accuracy) statement to improve reporting the quality of studies of diagnostic accuracy (17). Future studies for biomarkers should use these standards. The goal of these guidelines is to encourage transparent and complete reporting so that the relevant information will be available to others to help them to judge the usefulness of the data and understand the context in which the conclusions apply (17).

How can a biomarker be used in reproductive medicine?

We can learn from epidemiology on how to use biomarkers in the practice of clinical reproductive medicine. Standard epidemiologic research demonstrates that a biomarker can be used in at least four ways to (3): 1) Improve assessment of exposure; 2) Identify subgroups of different “susceptibility” to effects of treatment; 3) Measure early outcome with predictive significance; and 4) Differentiate subtypes with potentially different etiologies.

Improvement of assessment in exposure (or disease status) may include identification of “sub fertile” vs “infertile” patients or perhaps a gradation to account for “severity” of sub fertility. If successful, a marker may be able to help stratify when a couple is best served with expectant management or IVF. Identification of subgroups or “susceptibility” to effects

of treatment may aid in individualizing treatment for those who may need higher dose of medication or modification of standard laboratory conditions such as media or oxygen tension. Prediction of outcome may aid in early identification how many, or which embryo to transfer in IVF or prediction of miscarriage, ectopic pregnancy or obstetrical complications (such as preeclampsia, preterm labor or small for gestational age). Finally differentiation of subtypes with different etiologies may aid in differentiation of unexplained infertility or perhaps implantation failure.

Examples of categories of biomarkers with their intended clinical are presented in Table 1 (adapted 18). The categories of biomarkers in this table are grouped according to their purpose, following guidelines published by the FDA. Recently approved biomarkers from the FDA website from other therapeutic areas are provided as example, along with opportunities in development described in this issue of Fertility & Sterility or elsewhere. In this introduction, we will only cover 3 of the biomarker categories. The opportunities in reproductive medicine listed in table 1 are theoretical; at this time these biomarkers have not been validated.

Examples of biomarker research to improve therapeutic development & disease management

Disease diagnosis

The majority of studies published on a biomarker in reproductive biology deal with the diagnosis of disease. This includes, for example, diagnostics for infertility, polycystic ovarian disease, and endometriosis, location and viability of early pregnancy and others. In most examples the results point to individual markers of disease with very few examples of multivariate diagnostic markers. One of the first multivariate diagnostic tests to be developed and approved by the FDA was for OVA1 from Vermillion Inc (19). Its intended use is for adjunctive debulking surgery decisions for patients with ovarian adnexal masses. Recently Vodolozkaia et al. (20) have made progress towards a multivariate biomarker for non-invasive diagnosis of endometriosis.

The potential value of new biomarkers with their accompanying therapies to the endometriosis population has been estimated (Figure 2; adapted 20 and 21). A rather consistent estimate of the symptomatic endometriosis population ranges between 11 and 13 million women in the seven largest economies (US, UK, France, Spain, Italy, Germany, Japan) while there is an additional estimated 1 million asymptomatic US women (22). From the pool of potentially treatable, symptomatic women, only 8.5% receive a prescription for medication. This attrition from symptom to prescription is the result of imprecise diagnoses, and the undesirable side effects of existing therapies for this disease.

Development of a diagnostic that delivers an absolute improvement of 15% in the diagnosed population (from 44% to 59% correct diagnosis) in parallel with a drug therapy with reduced side effects and adverse events could be expected to increase therapeutic revenue several hundred million dollars per year. This represents significant opportunities in women’s health research.

Companion diagnostic / patient stratification

Table 2 reviews a portion of the biomarkers for which the FDA approved use of the biomarker as a companion in the drug label. The majority of tests identify patients that could have an adverse response to a drug. Among the 115 registered molecular genetic tests included on a drug label (22, 23), there are only three drugs that have an FDA approved pharmacogenomic biomarker described in their label for a reproductive endocrine use. The

contraceptive drospirenone, has identified interactions with CYP2C19 polymorphisms in vitro that were not confirmed in pharmacokinetic clinical studies; clomiphene, has a recommend Rh genetic test dating to its approval in 1967; and tolterodine (antimuscarinic drug used to treat urinary incontinence), has a recommended liver cytochrome P450 evaluation for patients with overactive bladder that have low levels of CYP2D6.

A forecast of emerging biomarker opportunities in reproductive medicine might include the use of genetic polymorphisms and molecular entities. Su et al has shown that a common single nucleotide polymorphism in *CYP3A4*, an enzyme in the cytochrome P450 system is involved in activating the chemotherapy agent cyclophosphamide, and it is associated with risk of ovarian failure in breast cancer patients undergoing chemotherapy (24). Rebbeck et al have shown that steroid hormone metabolism genotypes predict menopausal symptoms from hot flashes to depression (25). May et al (26) reviewed the existing list of biomarkers of endometriosis, and Burney and Giudice (27) reviewed the pathogenesis and pathophysiology of endometriosis from which new biomarkers may emerge.

Stratified molecular biomarkers may direct progesterone resistant patients to more effective treatments than progestin-based agonists or antagonists. Women stratified by deficiencies or compromises in macrophage surveillance of the peritoneal cavity, or of alterations in NK-cell cytotoxicity, may direct these patients towards immune therapies. If confirmed, these biomarkers would provide information for prediction of clinically important outcomes and allow physicians to individualize treatment plans.

In hindsight, two progesterone receptor modulators (asoprisnil and telapristone) have recently progressed through phase 2 clinical development and their development has been interrupted due to unexpected or adverse responses from a segment of the included patients. A subset of asoprisnil patients exhibited (28), cystic glandular dilation which could be differentiated from glandular hyperplasia. Telapristone (29) caused a dose-dependent safety event in a subset of patients that led to a postponement of this study by Repros and FDA (from clinicaltrials.gov). Development of a set of biomarkers that discriminate those patients that respond favorably from those patients that respond adversely to progesterone receptor modulators will be an important tool to identify patients with the maximal therapeutic benefit relative to health risk.

Brindsen et al. (30) found that recombinant leukemia inhibitory factor (LIF) failed to reduce recurrent implantation rather than the expected increase predicted by preclinical models. In hindsight, three lessons were learned. First, a stratification or inclusion test was not conducted in this study based on pre-existing LIF deficiency, LIF polymorphism, or a broader biochemical measure of endometrial failure. The pharmacogenetic test that was later identified (31, 32) has been questioned relative to the control population used to validate these markers (33). Companion diagnostic assays have subsequently been described (34, 35) that relied on a response to LIF, or were characterized by co-deficiencies of CLDN4 and LIF (36). Second, at the time of this trial there was not, and still does not exist, a panel of validated assays to distinguish the efficacy of LIF on local endometrial relative to peripheral immunological responses consistent with implantation in humans. Third, at the time this study was initiated, the viability and ploidy biomarkers for embryos were a covariate that was not controlled. Technologies in development may be more accurate at assessing embryo ploidy and viability biomarkers (37).

Biomarkers for Dose optimization

Biomarkers for dose optimization are linked with use of drug products from pharmaceutical companies (38). In the IVF field, Olivennes et al. (39) tested whether a clinically developed algorithm for the starting dose of recombinant FSH would affect the number of oocytes

retrieved from a first IVF cycle. The algorithm identified a lower starting dose for most patients (76.4%) than was traditionally prescribed by physician, while 9.9% of patients received the same dose and 13.7% received a higher dose. In this study, 97% of patients treated per protocol did not require dose adjustment during the IVF cycle, and the clinical pregnancy rate was higher in patients adhering to protocol (41.6%) than in the intent-to-treat population (34.2%). According to the biomarker validation process described above, this algorithm has yet to be confirmed in an independent set of patients. Assessments of embryo viability and endometrial receptivity have potential to become important biomarkers of the optimized ovarian stimulation treatment. While analysis of chromosomal ploidy was most obviously needed for women of advanced maternal age, the impact of controlled ovarian hyperstimulation on endometrial receptivity has highlighted the need for predictive biomarkers of implantation potential (40). Advances in the breadth of analytical technologies have now introduced a rather significant change in the way that pharmacogenomic tests can be applied in the ART laboratory.

Who are the new customers for emerging diagnostics and biomarkers?

The central lab pharmacogenetic business model (e.g. Qiagen; 2011 sales of $1.1 billion) has been cost effective, but the turnover from sample collection to sample analysis and results is longer than 24 hours. Nowadays, the introduction of customized assays for specialty clinical laboratories has the potential to drive new business towards the local medical offices. As an example, Qiagen, has partnered with Cardinal Health, a major health care distributor to make available molecular diagnostics products to approximately 5000 small and mid-sized hospitals in the U.S. (41). Although only 10 percent of US hospitals perform molecular diagnostics, the increasing use of diagnostic tests in hospitals suggests a new business opportunity for the development, registration and marketing of customized assays on simplified technology platforms for point-of-care specialty physician practices.

An example of this transition in IVF is aneuploidy determination. In the past, fluorescent in situ hybridization (FISH) for aneuploidy determination was conducted primarily in a central laboratory. The equipment and training was specific and not easily implemented in many IVF centers. Recent technology and methods developments have improved the scope of chromosomal interrogation (microarray platforms) and reduced the complexity of equipment required for analysis (37), thereby increasing the likelihood that aneuploidy assessment can become a point-of-care evaluation.

However, several diagnostic biomarkers in the IVF field are currently too complicated to be developed, clinically validated, and sold for point of care use. The financial value of specialized diagnostics (e.g. immunohistochemical staining) to the individual clinic (as their own marketing tool) may exceed the value that could be realized if the same clinic out-licensed the analyte to a diagnostic partner for subsequent point-of-care assay development. While technology has increased the easy and turnaround time for such potential marker, each will need to undergo validation and their utility assessed in properly designed clinical trials.

Conclusion

The concept of utility of a biomarker in clinical care is not new. In obstetrics the use of serum and ultrasound biomarker to assess the risk of aneupolidy in the fetus is standard of care. The use of biomarkers in reproductive medicine is less developed. The number of targets and potential use of biomarker is rapidly increasing. Many are presented in this issue of Fertility and Sterility. The science is outstanding and innovative. The breadth and the pace of new discovery are encouraging. Biomarkers are being developed for many potential

uses including; better identification of exposure (i.e. identification of aspects of both male and female aspects of infertility), to identify differences in susceptibility (who may be best treated and under what conditions), and to enhance prediction of outcome (which embryo should be transferred, or the location and viability of a gestation). However, much needs to be learned about the predicted significance of many of these proposed markers before any of these goals can be realized.

Extensive testing, validation and modification needs to be performed before a biomarker is demonstrated to have clinical utility. There are no short cuts. Unfortunately, the vast majorly of the markers describe in this special edition will not become part of clinical care. The proverbial home run that will instantly change clinical care is alluring. However, this kind of breakthrough in the development of diagnostics and therapeutics it is not as common as we would like (without the scientific equivalence of performance enhancing drugs).

When reading the following articles it is recommended that one considers the phase of development of each particular biomarker as well as the pathway to clinical practice. Finally, if a marker is near, or is ready, for clinical practice, its intended use, strengths and limitations should be clearly elucidated and understood. Education and understanding of the use and development of biomarkers is necessary to optimize its clinical utility and avoid misuse or over interpretation. As more biomarkers are moved into practice, a better educated biomarker consumer will enhance the possibility that biomarker(s) will realize their great potential.

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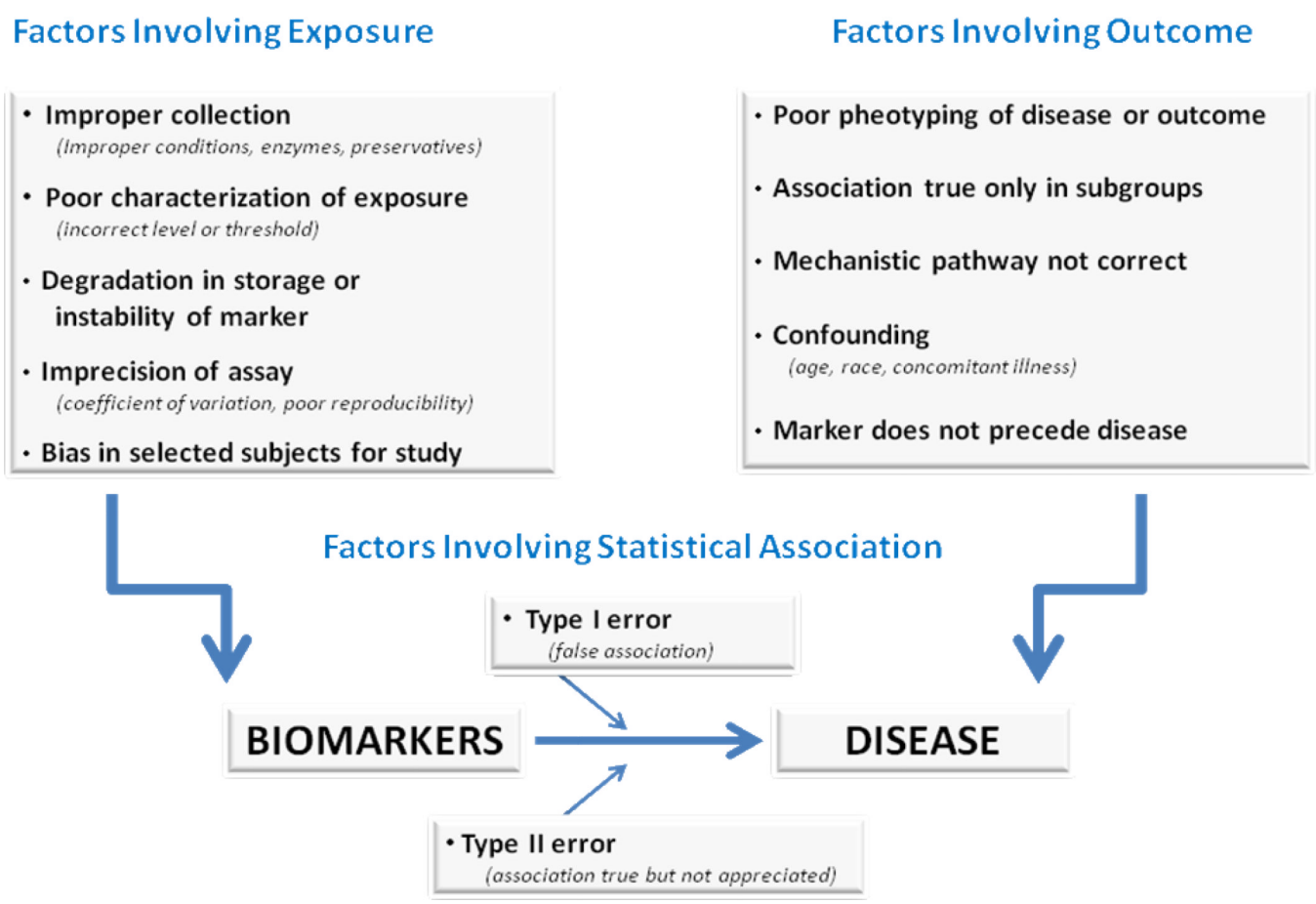

Figure 1.

For a biomarker to be valid it must have a strong and valid association with the disease process of interest. While initial studies of this association may be promising, there are many reasons that the association may not ultimately be demonstrated valid. Alternatively, there may be a true association that is overlooked or abandoned due to methodological factors. Factors can be categorized into issues involving the exposure, those regarding statistical associations, and those involving the outcome.

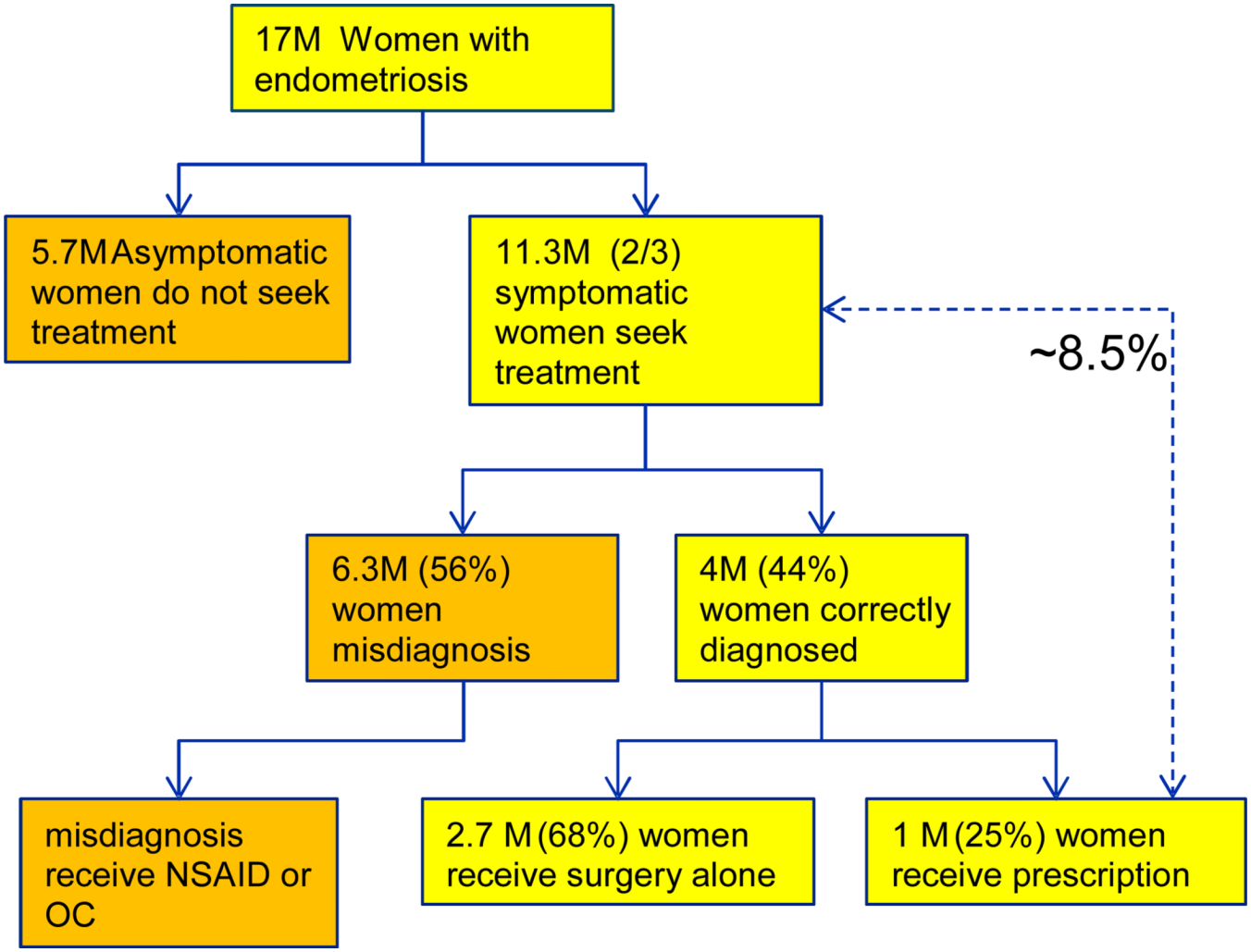

Figure 2.
Treatment of women with symptomatic endometriosis with surgery or pharmacologic therapy is currently impeded by the lack of validated diagnostics and the side effects of existing therapeutics.

Table 1

Summary of categories of biomarkers with examples from other therapeutic areas and opportunities in reproductive health

Biomarker Objective	**Intended Uses**	**Biomarker-based Decisions**	**Recent examples of FDA approved biomarkers from other therapeutic areas**	**Opportunities in Reproductive Health**
Disease diagnosis, staging, and prognosis	Classify disease stage with accuracy & precision	Identify therapy appropriate for stage of disease	OVA1 - 5 paramater multivariate test to further assess the likelihood that women with ovarian adnexal mass have malignancy present when the physician's independent clinical and radiological evaluation does not indicate malignancy.	Multivariate diagnostics for infertility, polycystic ovarian disease, endometriosis, location and viability of early pregnancy and others
Patient / subject stratification	Identify unique genetic or phenotypic traits that influence patient response to therapy	Establish inclusion / exclusion criteria for clinical trial Enrich patient populations with defined stratification criteria	The Roche AmpliChip CYP450 - identify a patient's CYP2D6 genotype from genomic DNA extracted from a whole blood sample, used as an aid to determine therapeutic strategy and treatment dose for therapeutics that are metabolized by the CYP2D6 gene product.	Stratification of endometriosis patients according to liver metabolism; progesterone resistance; immune disruption (23).
Dose optimization	Estimate effective dose in naïve patients or need for dose escalation based on clinical experience	No observed effect (NOEL) or adverse effect (NOAEL) in animal models Algorithm-based dose determination (quantitative algorithmic dosing)	Microgenics CEDIA Sirolimus assay −enables decisions to adjust Sirolimus dosage in patients with renal transplants	Orally active GnRH antagonists entering Phase III clinical trials; repurpose TNF-α inhibitors approved for rheumatoid arthritis into endometriosis
Toxicity minimization	Predictor of onset of toxicity Predictor of reversal of toxicity	Reduce adverse response to therapy, Decision tool for length of patient monitoring	Stratify Jcv Antibody ELISA; used to identify patients at risk for multifocal leukoencephalopathy while on treatment for multiple sclerosis or Crohn' disease	Progesterone antagonists
Mechanism of action	Most feasible proximal marker of drug effect is affected at time when drug is bioavailable	Is the biochemical target modified by therapy?	Covidien OxiMax N-600x Pulse Oximeter with SPD–used to monitor SpO2 content in patients with repetitive reductions in airflow to lungs	Markers of endometrial status in the intrauterine fluid and immune cells compared to peripheral immune cells
Response to therapy	Distal marker of drug effect predicts treatment outcome	Early predictor of efficacy prior to disease modifying response	Bayer Contour Next Blood Glucose Meter –used to measure blood glucose response to diabetes therapy	Markers of regression of endometriotic lesions or resolution of a tubal pregnancy.

Table 2

Molecular or genetic biomarkers for which the FDA approved the use of the biomarker as a;companion in the drug label.

Therapeutic area	# biomarker tests	Intended Use	Example
Oncology	10	Reduce exposure of drug to patients with hepatic cycP450 polymorphism at risk for adverse response	Irinotecan; UGT1A1*28 allele
	20	Identify patients with mutations likely to respond and preferred Dosage	Imatinib; mutations in PDGFR gene, D816V c-kit, FIP IL-1 PDGFR-alpha fusion
Oncology - stratification	14	Confirm presence of mutation before initiation of therapy	Herceptin; HER-2/Neu antibody for c-erbb-2 antigen, Cetuximab; therapy recommended for patients without KRAS mutation
Psychiatry	30	Identify hepatic cycP450 polymorphisms to avoid drug-drug interactions	Risperidone; CYP2D6 polymorphisms associated with listed drug interactions and clinical pharmacology
Reproductive endocrinology	3	Identify hepatic cycP450 polymorphisms to avoid metabolic safety risk	Tolterodine; Level of hepatic CYP2D6 expression